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# **Nitrous oxide release from agricultural riparian ecosystems**

A thesis submitted for the degree of Doctor of  
Philosophy

by

**Séverine Machefert**

**Diplome Universitaire de Technologie 1999**

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## Abstract

The closed chamber technique and acetylene inhibition method were applied to the investigation of the environmental factors controlling nitrous oxide ( $\text{N}_2\text{O}$ ) emissions in the field and denitrification, both *in situ* and in the laboratory, from agricultural riparian ecosystems.

$\text{N}_2\text{O}$  emissions were measured along with environmental factors weekly to fortnightly over a whole year and were found to be mainly controlled by water-filled pore space (WFPS) and soil temperature with a threshold response at 35% WFPS and  $8^\circ\text{C}$ , below which  $\text{N}_2\text{O}$  emissions were very low. Nitrate ( $\text{NO}_3^-$ ) was not a limiting factor at either of the two experimental sites. There was also a 'threshold' effect of rainfall, in which major rainfall events ( $\geq 10\text{mm}$ ) triggered a pulse of high  $\text{N}_2\text{O}$  emission if none of the other environmental factors were limiting.

The best model for denitrification in riparian ecosystems included water-filled pore space as the main explanatory variable and soil nitrate. Denitrification rates were measured in an intact riparian site and were exponentially correlated to the water-filled pore space of the soil. A threshold response at 60-80% WFPS was also found. The absolute denitrification rate was also related to the soil  $\text{NO}_3^-$  concentration. Annual denitrification fluxes were determined on different levels of the riparian zone and were  $5.0$  and  $4.8 \text{ kg N ha}^{-1}$  at the intermediate and upper levels respectively, farthest from the stream surface where the moisture status of the soil was not significantly different and  $71.7 \text{ kg N ha}^{-1}$  at the near-stream site where the soil moisture was higher. These results were used to calibrate the INCA model and run an advance version of the model, INCA-N Riparian in order to predict denitrification rates in riparian ecosystems. Annual denitrification rates were well simulated in response to moisture changes.



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# Chapter One

## Introduction

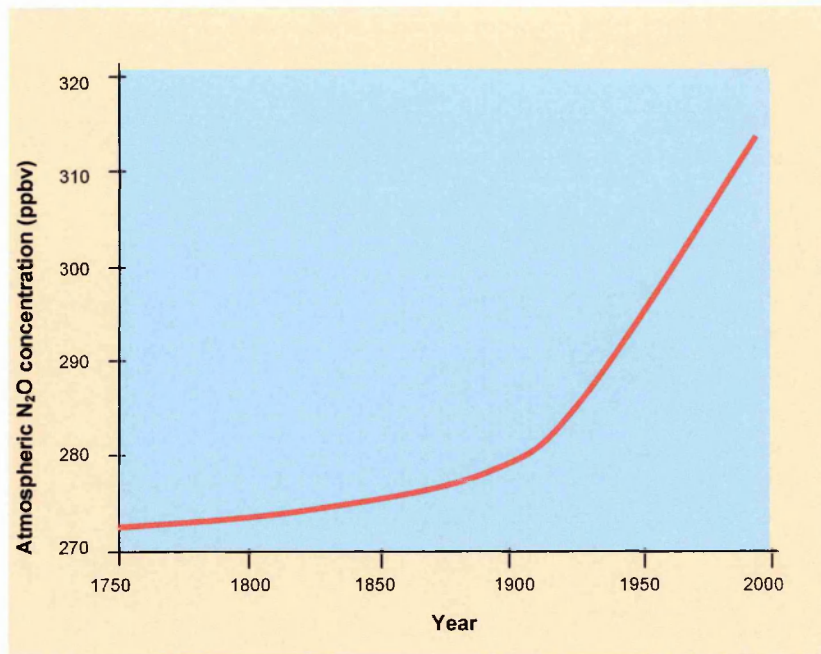
(Parts of this chapter are published in: Machefert, S. E., Dise, N. B., Goulding, K. W. T. and Whitehead, P. G., Nitrous oxide emission from a range of land uses across Europe, *Hydrology and Earth System Sciences*, 2002, 6(3), 325-337)

### 1.1 General Overview

Nitrogen sources, sinks and hydrological transport through ecosystems affect the vitality of ecosystem functioning and are impacted by humans (Wade *et al.*, 2002). The utilisation of nitrogenous fertilisers in agriculture and nitrogen emissions from industry leads to increasing atmospheric pollution that affects ecosystems through, for example, their nitrate and ammonium loadings. Diverse effects include acidification of soil, streams and lakes (Skeffington and Wilson, 1988) or eutrophication of surface- and ground-water as a result of contamination with nitrate. In addition to these anthropogenic inputs, vegetation as well as mineralization and nitrification of organic N in soils contribute to the nitrogen inputs to river systems.

Nitrous oxide (N<sub>2</sub>O) contributes 4 to 6 % to the anthropogenic enhancement of the greenhouse effect and has increased, since the Industrial Revolution, by about 16 %, from a pre industrial value of about 270 ppb to 314 ppb at present (Fig.1.1). Although

the concentration of  $\text{N}_2\text{O}$  is about 100 times less than carbon dioxide ( $\text{CO}_2$ ) one molecule of  $\text{N}_2\text{O}$  absorbs 270 times more radiation than a molecule of  $\text{CO}_2$  and its global warming potential over a 100 year time horizon is 310 times greater than that of  $\text{CO}_2$  (IPCC, 1996). The global warming potential is the index used to translate the level of emissions of various gases into a common measure in order to compare the relative radiative forcing of different gases without directly calculating the changes in atmospheric concentrations. Global warming potentials are calculated as the ratio of the radiative forcing that would result from the emissions of one kilogram of a greenhouse gas to that from emission of one kilogram of carbon dioxide over a period of time (usually 100 years). Once emitted, the  $\text{N}_2\text{O}$  molecule drifts throughout the lower atmosphere, possibly for decades, until it enters the lower stratosphere where it is broken down by ultraviolet light into O, N or NO. The NO is then available to participate in the destruction of stratospheric ozone (Crutzen, 1970).



**Figure 1.1:** Increase in atmospheric nitrous oxide concentration since the 18<sup>th</sup> century (from analysis of the H15 ice core collected in Antarctica, Machida *et al.*, 1995).

N<sub>2</sub>O is naturally produced, mainly by microbes in oceans and soils, and anthropogenic sources include agriculture, industry and livestock management (Table 1.1). About 70 % of the total emitted N<sub>2</sub>O is derived from soils (Bouwman, 1990) and agriculture as a whole (i.e. animal excreta, denitrification of leached nitrate, etc) contributes c 81 % of the anthropogenic N<sub>2</sub>O emissions (Brown *et al.*, 2001).

Base year	1994 (Tg N yr <sup>-1</sup> / % of total)	range (Tg N yr <sup>-1</sup> )
<i>Sources</i>		
Ocean	3.0 / 17	1 – 5
Atmosphere (NH <sub>3</sub> oxidation)	0.6 / 3.4	0.3 – 1.2
Tropical soils		
Wet forests	3.0 / 17	2.2 – 3.7
Dry savannas	1.0 / 5.6	0.5 – 2.0
Temperate soils		
Forests	1.0 / 5.6	0.1 – 2.0
Grasslands	1.0 / 5.6	0.5 – 2.0
<i>All soils</i>		
Natural sub-total	9.6 / 54	4.6 – 15.9
Agricultural soils	4.2 / 24	0.6 – 14.8
Biomass burning	0.5 / 2.8	0.2 – 1.0
Industrial sources	1.3 / 7.3	0.7 – 1.8
Cattle and feedlots	2.1 / 12	0.6 – 3.1
Anthropogenic sub-total	8.1 / 46	2.1 – 20.7
Total sources	17.7 / 100	6.7 – 36.6

**Table 1.1:** Estimates of the global nitrous oxide budget (in Tg N/yr) from different sources. Data are from Mosier *et al.* (1998) and Kroeze *et al.* (1999).

N<sub>2</sub>O is both emitted and absorbed by soils, but the net flux is almost always emission. Due to seasonal and spatial variation, N<sub>2</sub>O emissions are difficult to quantify (Smith *et al.*, 1994) and the estimation of annual emissions from a small number of observations may lead to considerable errors. This emphasises the need for long-term studies to overcome the problem.

## 1.2 Processes

Two mechanisms are mainly responsible for  $\text{N}_2\text{O}$  emissions from soils, both microbially mediated: nitrification and denitrification.

Nitrification is the oxidation of ammonium ( $\text{NH}_4^+$ ) to nitrite ( $\text{NO}_2^-$ ) and then nitrate ( $\text{NO}_3^-$ ). It is an aerobic process carried out by a few genera of autotrophic bacteria able to use the energy generated from these processes. The best studied are the obligate chemoautotrophs, *Nitrosomonas* and *Nitrobacter* species (Robertson and Kuenen, 1991). At sub-optimal oxygen concentrations, oxidation to  $\text{NO}_3^-$  is incomplete and some of the  $\text{NH}_4^+$  is channelled into the production of NO and  $\text{N}_2\text{O}$  (Poth and Focht, 1985). Bremner and Blackmer (1981) report that  $\text{N}_2\text{O}$  production is higher with added nitrifiable nitrogen (e.g. urea or ammonium containing fertiliser). Nitrification occurs most rapidly when soil pH is between 5.5 and 6.5 (Kasica, 1997). For instance, nitrification rates from pasture soils were found to be higher in the zone of the soil with a pH value of 5.7 than in a deeper soil layer with a pH value of 4.7 (Black *et al.*, 1998). In the field, the rate of nitrification is also affected by the moisture content and temperature of the soil (Table 1.2).

Denitrification is the anaerobic process by which nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) are reduced to give nitric oxide (NO), nitrous oxide ( $\text{N}_2\text{O}$ ) and dinitrogen ( $\text{N}_2$ ). It requires a ready supply of reduced carbon for energy and  $\text{NO}_3^-$  as a substrate. A wide range of micro-organisms can denitrify. They are facultative anaerobes and switch to  $\text{NO}_3^-$  as a terminal electron acceptor when oxygen is unavailable. Important environmental controls for denitrification include temperature, soil moisture and pH (Table 1.2). Denitrification will have different products depending on the level of soil moisture, with NO favoured by lower soil moisture, grading into  $\text{N}_2$  favoured at the highest soil moisture. However, it is still unclear exactly what level of soil moisture will lead to primarily NO,  $\text{N}_2\text{O}$  or  $\text{N}_2$ .

	Nitrification	Denitrification
Substrate availability	NH <sub>4</sub> <sup>+</sup> , urea, amino acids	NO <sub>3</sub> <sup>-</sup>
O <sub>2</sub> concentration	high	low
Reduced carbon	no effect	high (energy source)
Moisture (water filled pore space)	intermediate 30-70 % WFPS	high 55-100 % WFPS
Soil temperature	high (within range)	high (within range)
pH	> 5	Low (< 5)

**Table 1.2:** Factors favouring nitrification and denitrification processes

The extent to which these two processes, nitrification and denitrification, contribute to N<sub>2</sub>O emission varies with climate, soil conditions and soil management. Generally, high rainfall, poor drainage, fine soil texture and high organic carbon content promote denitrification whereas low rainfall, good drainage and aeration and coarse texture promote nitrification (Groffman, 1991). However, due to the complex interactions of the factors influencing the processes, it is difficult in most soils to determine which process prevails and what proportion of the nitrogen released is N<sub>2</sub>O. The processes of denitrification and nitrification can also co-occur at the same time in a single site due to micro-scale soil heterogeneity, and the balance between the two processes can switch very rapidly (Smith, 1980; Kuenen and Robertson, 1994).

A third, not well known process has recently been studied by *Wrage et al.* (2001): nitrifier denitrification. This process is carried out by autotrophic nitrifiers that oxidize

ammonia ( $\text{NH}_3$ ) to nitrite ( $\text{NO}_2^-$ ) and then reduce  $\text{NO}_2^-$  to nitric oxide (NO), nitrous oxide ( $\text{N}_2\text{O}$ ) and molecular nitrogen ( $\text{N}_2$ ). Nitrifier denitrification can lead to substantial  $\text{N}_2\text{O}$  emissions especially when low oxygen conditions are coupled with low organic carbon contents of soils and low pH.  $\text{N}_2\text{O}$  lost via nitrifier denitrification in soils could represent as much as 30% of the total  $\text{N}_2\text{O}$  production (Webster and Hopkins, 1996), but remains an extremely poorly understood process.

### **1.3 Nitrous oxide emissions from a range of land-uses across Europe**

#### **1.3.1 Introduction**

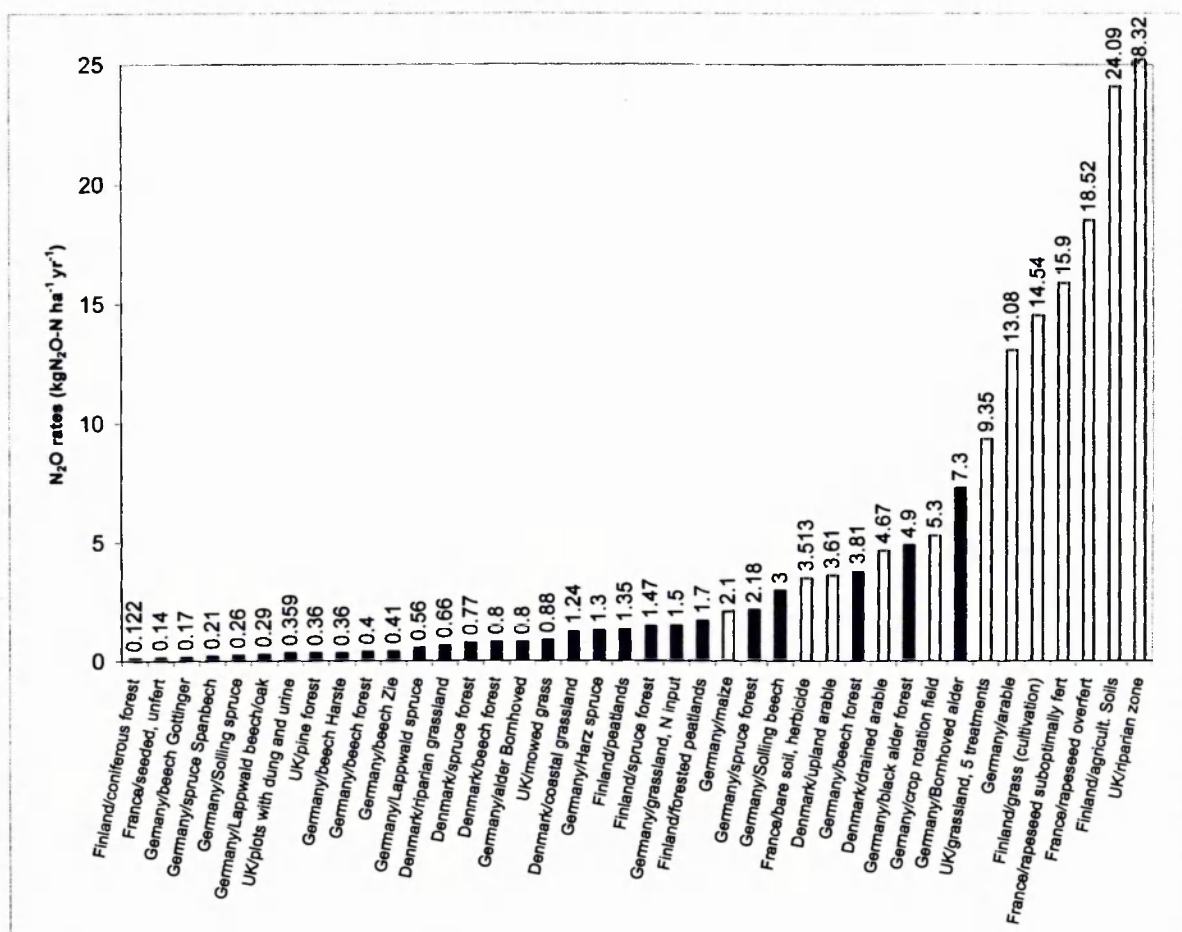
Nitrous oxide emissions have been studied for decades in most types of ecosystems. The data presented here are from a major European Union 5<sup>th</sup> Framework project. The purpose of the project was to establish the factors controlling mitigation of nitrogen pollution to a river system by release of gaseous N, and thereby refine the denitrification equation in the Integrated Nitrogen in European CAtchments (INCA) model (Whitehead *et al.*, 1998a). The expected output is a model of annual  $\text{N}_2\text{O}/\text{N}_2$  flux from riparian zones as related to the key controlling processes and an increased understanding of the contribution of nitrous oxide emissions from a range of land-uses across Europe. The analysis was made by compiling data from reviews and site-specific field experiments on  $\text{N}_2\text{O}$  flux controls for a variety of European ecosystems (Appendix I). The 33 individual experiments from 13 references were from grasslands (fertilised or non-fertilised), forests (subjected to variable amounts of atmospheric N deposition) and agricultural sites (different crop types) across Europe. Only studies with at least 1 year's data are included. All of the 13 references used the closed chamber method for determining  $\text{N}_2\text{O}$  emission rates (Hutchinson and Mosier, 1981), and gas



samples were then analysed by gas chromatography. The standard soil- and weather-dependent parameters were measured in most cases. These include precipitation, air temperatures, soil temperatures at different depths (digital thermometer), water-table levels, and pH of soil in water. Soil water content was determined in most cases and results were given either as volumetric water content, gravimetric water content (mass/mass) or as water-filled pore space. Soil analyses for nitrate and ammonium concentrations (extractions with  $\text{CaCl}_2$  in Flessa *et al.*, 1995;  $\text{KAl}(\text{SO}_4)_2$  in Papen and Butterbach-Bahl, 1999 or KCl in other references) were also performed in all studies but three: Burt *et al.* (1999) and Nieminen (1998) determined nitrate and ammonium concentrations in ground-water samples; Martikainen *et al.* (1994) made no determinations. Water-soluble organic carbon compounds were determined by the method described in Burford and Bremner (1975) and the soil organic matter as Loss-On Ignition method.

### *1.3.2 Results and discussion: overall patterns of $\text{N}_2\text{O}$ emissions*

The data compilation indicates a gradient of  $\text{N}_2\text{O}$  emissions with low fluxes for forests and grasslands, and higher emissions from agricultural fields (Figure 1.2). Five forested sites in Germany show  $\text{N}_2\text{O}$  emissions within the same range obtained for the arable agricultural sites; these are Höglwald (two sites), Solling, Schelswig-Holstein and Bornhöved. Germany is among the European countries receiving the highest atmospheric N deposition as oxidised or reduced nitrogen. The mean annual precipitation for these regions is about 850mm. The soils are acidic and mostly organic.



**Figure 1.2:** Nitrous oxide emission rates for different European ecosystems – Black bars represent forested and grassland ecosystems. White bars represent agricultural ecosystems.

Brumme *et al.* (1999) report a study of eleven forest ecosystems in Germany comprising mainly alder, beech and spruce (Table 1.3, from Machefert *et al.*, 2002). Element budgets and soil characteristics were measured in these forests and showed distinct differences between sites, including pH ranging from 3.6 to 5.6. Nitrogen deposition ranged from 20 to 41 kg N ha<sup>-1</sup> yr<sup>-1</sup>. These ecosystems have different soil covers including a fluvial layer of sandy clay loam. Nitrous oxide emissions were measured weekly or biweekly over one year with closed chambers.

Site location	Vegetation	N <sub>2</sub> O emission kg N <sub>2</sub> O-N ha <sup>-1</sup> yr <sup>-1</sup>	Soil Bulk density (0-5 cm) g cm <sup>-3</sup>	Precipitation mm yr <sup>-1</sup>	N <sup>c</sup> deposition kg N ha <sup>-1</sup> yr <sup>-1</sup>	Type of flux
Bornhoved (d)	alder	7.3	0.48	697	33	s
Solling	beech	3.0	1.01	1090	35	s
Harz	spruce	1.3	1.2	1239	20	e <sup>a</sup>
Bornhoved	alder	0.80	-	697	33	b
Lappwald	spruce	0.56	1.2	650	-	b
Zierenberg	beech	0.41	0.75	700	21	b
Harste	beech	0.36	1.17	750	26	b
Lappwald	beech/oak	0.29	0.85	650	-	b
Solling	spruce	0.26	0.91	1090	41	b
Spanbeck	spruce	0.21	1.01	650	31	b
Gottinger Wald	beech	0.17	0.79	680	28	b

Data from Brumme *et al.* (1999)

(d) – drained

<sup>a</sup> Background fluxes were also observed at this site

<sup>c</sup> Throughfall of NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup> + N<sub>org</sub>

s = 'seasonal'; b = 'background'; e = 'event-based'

**Table 1.3:** Annual losses of nitrous oxide and some site characteristics

Brumme *et al.* (1999) distinguished three types of emission patterns determined by the differences in temporal variation: (a) 'seasonal' emission pattern, (b) 'event-based' emission pattern and (c) 'background' emission pattern. The 'background' pattern is characterised by low annual fluxes. They found that most sites show background emission patterns, with low emissions during the whole year and low annual site means ranging from 0.17 to 0.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> (Table 1.3). Similar and relatively constant N<sub>2</sub>O emissions were found in one forest in Finland (Martikainen *et al.*, 1994), three forests in the UK (Skiba *et al.*, 1996), one other forest in Germany (Mogge *et al.*, 1998) and two forests in Denmark (Ambus and Christensen, 1995), with annual emissions ranging from 0.12 to 0.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> (Appendix I). Only two of the sites studied by Brumme *et al.* (1999) appeared to display 'seasonal' patterns. Such sites are characterised by a period of elevated rates in summer. These two sites had much higher annual fluxes: 3.0 and 7.3 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> (Table 1.3). Some of the forested sites listed in Appendix I show similar fluxes. The 'event' emission pattern is characterised by short peaks of N<sub>2</sub>O emission during or following periods such as frost

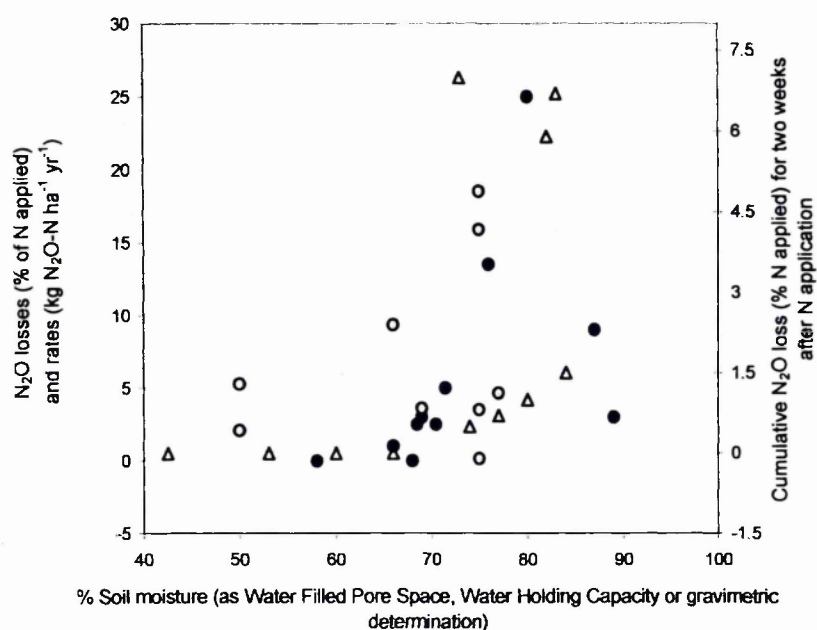
or thaw. Brumme *et al.* (1999) observed this type of emissions at a drained site in Germany, with  $\text{N}_2\text{O}$  flux changing from  $8.8 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$  to about  $43.8 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$  with the onset of the spring thaw in 1996.

Nitrous oxide emissions from soils have been widely studied in the past decades and it is generally agreed that the main processes responsible for emissions, namely nitrification and denitrification, are not controlled by only one parameter but by several interacting parameters, making predictions very difficult.

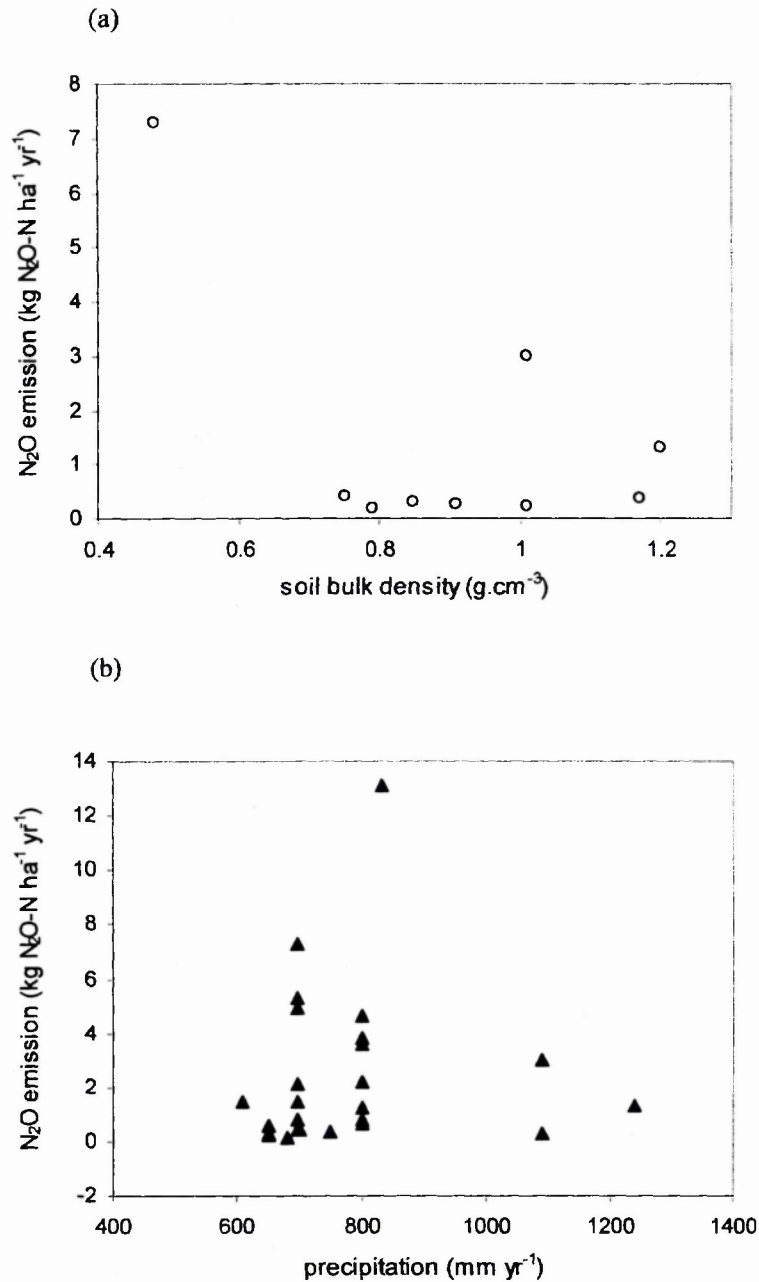
#### SOIL MOISTURE, BULK DENSITY, RAINFALL

Hydrological factors seem to exert the strongest controls on annual  $\text{N}_2\text{O}$  emissions for sites in the compilation (Figure 1.3). These factors affect nitrification and denitrification in different ways. Denitrification will be favoured by high moisture contents whereas nitrification will occur in drier soils. For instance, it has been observed (Davidson, 1991) that nitrification is the dominant source of  $\text{N}_2\text{O}$  when water-filled pore space (WFPS, calculated using gravimetric water content) is less than 60% and that denitrification is the predominant source when WFPS is greater than 60%.

No obvious relationship was found between  $\text{N}_2\text{O}$  emissions and bulk density (Figure 1.4 a) due to few data available. Figure 1.4 (b) shows a plot of  $\text{N}_2\text{O}$  emissions against mean annual precipitation and suggests  $\text{N}_2\text{O}$  emissions increase above a threshold value (around  $650 \text{ mm yr}^{-1}$ ) of precipitation when precipitation is below  $800\text{-}1000 \text{ mm yr}^{-1}$ . However, more data are required to ascertain this situation.



**Figure 1.3:** Relationship between annual nitrous oxide emissions and soil moisture. (●) Dataset for fertilised sugar cane, banana and pasture in the tropics of Costa Rica. The data were redrawn from Veldkamp *et al.* (1998). (Δ) Data points from managed grassland in W. Europe (Dobbie *et al.*, 1999). Grey circles represent the data from agricultural soils in Europe (see studies in Appendix I). The scale for the N<sub>2</sub>O data from the tropical soils and from the agricultural data is on the left side of the graph, from the grassland data on the right side of the graph.



**Figure 1.4:** Relationships between annual nitrous oxide emissions and soil bulk density (a), and annual precipitation (b). (▲) All data (13 references from Appendix I and Brumme *et al.*, 1999). (○) Data points from Brumme *et al.*, 1999.

High N<sub>2</sub>O emissions measured by Brumme *et al.* (1999) took place when the soil water content was near field capacity (~1kPa) and lasted until the soil water suction reached about 2kPa. Once this threshold was reached, N<sub>2</sub>O emissions decreased. However, during the same period low background emissions were observed at other sites as well. In the study of Skiba *et al.* (1996) daily and even seasonal changes in moisture

were not very well correlated with  $\text{N}_2\text{O}$  fluxes. However, they observed a strong correlation between annual precipitation and annual  $\text{N}_2\text{O}$  fluxes. Their data for a coniferous forest in central Scotland give some clues about the relative importance of temperature and soil moisture. They observed, for the same soil, that wetter soil at lower temperatures had higher fluxes than drier soil at higher temperature ( $0.47 \text{ kg N ha}^{-1}$  with a mean soil moisture content of 34% of soil dry weight and average soil temperature of  $10^\circ\text{C}$  for 1993; and  $0.3 \text{ kg N ha}^{-1}$  with a mean soil moisture content of 25% of soil dry weight and average soil temperature of  $12^\circ\text{C}$  for 1994). Mogge *et al.* (1998), in their study of two forest sites in Höglwald, Germany, show that an increase in soil moisture, due to precipitation, contributed to the high  $\text{N}_2\text{O}$  emissions observed at both sites (precipitation recorded from July to September and in December). A similar positive correlation was reported in another of their studies (Mogge *et al.*, 1999). The literature also suggests that a threshold for soil gravimetric water content of about 60-70% exists above which significant  $\text{N}_2\text{O}$  emission can occur. In temperate climates (Dobbie *et al.*, 1999) as well as in the tropics (Veldkamp *et al.*, 1998), maximum  $\text{N}_2\text{O}$  emissions have been found to occur at a water filled pore space (WFPS) of 75-85% (mass/mass, Fig.1.3). Maximum  $\text{N}_2\text{O}$  emissions have also been found when soil moisture increased (e.g. during precipitation events) in forest soils and potato fields (Mogge *et al.*, 1998; Ruser *et al.*, 1998). As well as regulating the emission rate, water filled pore space regulates the proportion of  $\text{N}_2\text{O}$  emission from nitrification and denitrification due to its effect on  $\text{O}_2$  diffusion. Nitrous oxide and NO losses are both high in poorly aerated soils and only in very poorly aerated soils (waterlogged soils with Eh close to 0V) does  $\text{N}_2$  emission dominate.

These studies clearly show that soil moisture influences  $\text{N}_2\text{O}$  emissions whereas rainfall events show no clear relationship with daily fluxes. This may be a result of the different response soils have to rainfall according to their nature. For instance, where soils contain higher percentages of clay, diffusion of the water through the soil will be slower and high rainfall will not necessarily result in immediate higher soil moisture

content. Also, part of the rainfall will be accounted for as runoff water. However, differences in mean annual rainfall had a significant effect on the total annual  $\text{N}_2\text{O}$  emissions.

#### N INPUT, N OUTPUT, N FERTILISATION, N SATURATION

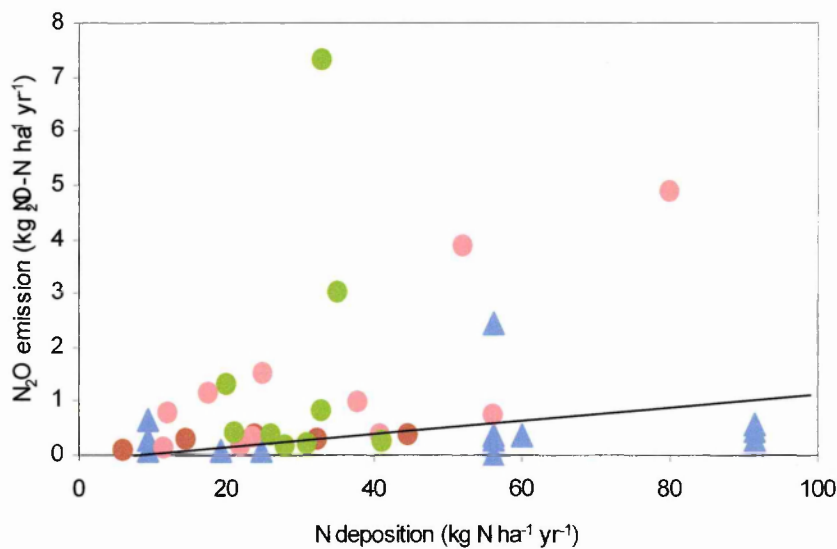
Nitrogen availability is another control for nitrification and denitrification, but different forms of inorganic N will have different effects:  $\text{NH}_4^+$  availability will influence nitrification, and denitrification will be affected by  $\text{NO}_3^-$  availability (Table 1.2). However, the two processes are closely linked since  $\text{NO}_3^-$  ions are produced by nitrification of  $\text{NH}_4^+$ . It is still unclear what the minimum concentrations for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  are below which denitrification or nitrification will not occur.

Data on forested ecosystems in Europe (NITREX, Matzner, 1989) showed that N deposition affects the excess of nitrogen in the soil solution. Brumme *et al.* (1999) did not find any effect of N deposition on  $\text{N}_2\text{O}$  emissions from sites with background emissions, presumably because N deposition did not result in excess mineral N in the soil. However, two sites showed high  $\text{N}_2\text{O}$  emissions in the summer (the 'seasonal' emissions sites, Solling Beech and Bornhöved). N deposition to the Solling sites is very high, but it is unclear why high  $\text{N}_2\text{O}$  emissions were found at the Solling beech stand but not the spruce stand. Quantity and quality of labile carbon is one likely factor, however it seems to only have a secondary effect. In the case of the drained alder forest in Bornhöved it could be explained by the fact that alders are N-fixing species which can exude nitrate into the soil from their nodules and also produce leaf litter with a high N content.

Emission factors are a statistical average of the rate at which a pollutant is released to the atmosphere as a result of some activity divided by the rate of that activity (Stern, 1977). The Intergovernmental Panel on Climate Change (1997) estimated that 1% of the N supplied by atmospheric deposition to natural soils is emitted as  $\text{N}_2\text{O}$ . This is a



simple estimate (or 'emission factor' or 'default value') based on input data readily available from the FAO databases. In Fig. 1.5, the data points significantly above the 1% IPCC default line are from sites which had received continuous elevated N deposition rates for many years. Many of the values below this line but receiving high N deposition are from field experiments where elevated N deposition was simulated for a relatively short time.



**Figure 1.5:** N deposition induced emissions from forest and moorland soils. In upland areas (brown circles), large-scale acid mist experiments (blue  $\Delta$ ), downwind of point sources: poultry and pig farms (pink circles), and German forests (green circles), IPCC emission factor 1% (solid line). Data redrawn from Skiba and Smith, 2000 and Brumme *et al.*, 1999

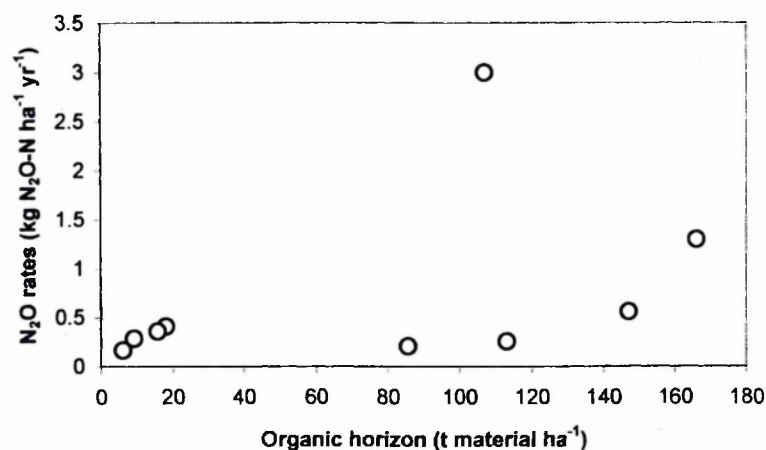
In the sites studied by Brumme *et al.* (1999), the minimum N deposition is about 20 kg N ha<sup>-1</sup>yr<sup>-1</sup> (Table 1.3). Applying fertiliser only seemed to generate pulses of N<sub>2</sub>O emission but showed no long-term effect. Results for agricultural sites from Skiba *et al.* (1996) showed a positive response of N<sub>2</sub>O emissions a few weeks after fertilisation. This was also observed by Mogge *et al.* (1999). Moreover, the timing of fertiliser application appears to be an important factor affecting annual fluxes, with higher

annual N<sub>2</sub>O fluxes if fertiliser is applied during warmer months. Major increases in N<sub>2</sub>O flux can occur shortly after fertilisation, with near background emissions restored within several weeks after application (Skiba and Smith, 2000).

Together, the N-deposition and N-fertilisation data suggest that the 'N status' of the sites, i.e. the availability of mineral N substrate for nitrification and denitrification (applied, or derived from organic N applied), is probably a secondary control for N<sub>2</sub>O emissions after moisture and temperature. However, N<sub>2</sub>O emission will only occur if a minimum level of N substrate is present in the sites. It may also be that N<sub>2</sub>O losses will increase rapidly with N input once the system has reached optimum levels of the other factors controlling N<sub>2</sub>O emission.

#### CARBON SOURCE, LITTER QUALITY, CROP TYPE

The availability of labile carbon is an important control for denitrification. In their study, Brumme *et al.* (1999) looked at the effect on annual N<sub>2</sub>O emissions of the mass of the organic horizon in the soils studied (Fig. 1.6). There are not enough data to draw definite conclusions, but an increase in the mass of the upper organic horizon may well provide an enhanced carbon source for nitrification/denitrification for which it might be possible to determine a threshold with more data. Nitrous oxide fluxes may be higher for organic upper horizon >100 t.ha<sup>-1</sup>. However, it is unclear why of the well drained forests considered in the study of Brumme *et al.* (1999; Fig. 1.6) only the beech forest in Solling has seasonal emission patterns. Correlations between annual emissions of N<sub>2</sub>O and state variables of the sites were only weak (Figs. 1.4 and 1.6). This is to be expected since only the beech stand in Solling had high emissions but comparable state variables compared to the other stands. The strong non-linear relationships in Figures 1.4 and 1.6 probably indicate that more than one state variable are responsible.



**Figure 1.6:** Relationship between annual nitrous oxide rates and the total mass of material in the organic upper horizon. Data from Brumme *et al.*, 1999

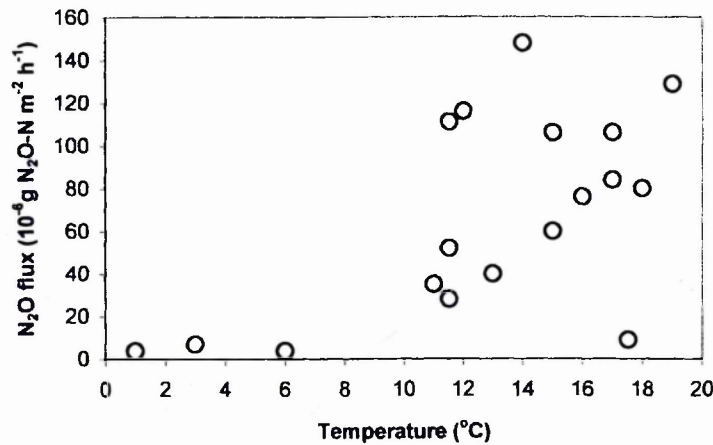
Different crop types appear to emit different amounts of N<sub>2</sub>O. This has been shown by Skiba *et al.* (1996) in their study of a range of agricultural and semi-natural soils in south and central Scotland. For instance, a potato crop emitted more N<sub>2</sub>O than cereals. An explanation for this was the contribution of more labile crop residues following harvest, and root exudation during tuber development. Dobbie *et al.* (1999) obtained higher emission factors from potato and brassica crops (1.8-7% of N applied) than for wheat and barley (0.2-0.7% of N applied). This was also found by Henault *et al.* (1998) for oil seed rape compared to wheat (0.55 compared to 0.42%). The reasons for differences in N<sub>2</sub>O emissions according to crop type are primarily related to the crop requirements for specific climatic conditions and management. Similar observations have been made by Brumme *et al.* (1999) regarding the litter quality in forested ecosystems. In an experiment where litter fall between beech and spruce stands was exchanged (Solling, Germany), N<sub>2</sub>O emissions increased in the spruce stand after application of beech litter and decreased in the beech stand after spruce litter had been applied. However, the change in N<sub>2</sub>O flux between controls and treated plots was much

less than the actual differences between control stands, suggesting either that a longer time is needed to obtain a flux response or that other factors are important.

## TEMPERATURE

Both nitrification and denitrification rates are controlled by soil temperature. The rapid increase in process rates with increasing temperature suggests that the microbial response to temperature is primarily a biochemical response rather than a population one. Thus, temperature is a fast response parameter. Seasonal and diurnal changes in temperature have been shown to be correlated with N<sub>2</sub>O emission for many soils in temperate climates (Skiba *et al.*, 1998; Skiba and Smith, 2000). But this is only true when other important factors such as WFPS or mineral N are not limiting. This was shown by Dobbie *et al.* (1999) in their study of intensively managed agricultural fields, with Q<sub>10</sub> values of up to 8. The Q<sub>10</sub>-value or temperature coefficient is defined as the change in the rate of a process as a result of increasing the temperature by 10°C. In their study of 11 forest soils in Germany, Brumme *et al.* (1999) observed an increase of the N<sub>2</sub>O emission from 6 µg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> up to a more or less constant level of about 90 µg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> if soil temperature exceeded 10°C. Their data also indicated that during the period of high emissions, N<sub>2</sub>O fluxes followed changes in temperature. The Q<sub>10</sub>-values obtained for this study were as high as 14. Such an extremely high Q<sub>10</sub> is partially explained by temperature-induced positive feedback. For instance, a rise in temperature will have an effect on soil respiration and anaerobicity thus influencing nitrification and denitrification rates (Smith, 1997). In addition, the data obtained by Brumme *et al.* (1999) show that N<sub>2</sub>O fluxes are related to the air temperature (e.g. Solling Beech site, Fig. 1.7) with small fluxes at temperatures below 8°C and larger fluxes more likely to happen at higher temperature, but depending on other factors. For the study period, the temperature ranged between -7.5°C and 25°C. N<sub>2</sub>O fluxes will also be related to the soil temperature since the soil temperature is related to air

temperature but is lagged with time and damped with depth. For the *Brumme et al.* (1999) study, only air temperature was available.



**Figure 1.7:** Relationship between air temperature and N<sub>2</sub>O flux at the Solling beech site. Data redrawn from Brumme *et al.*, 1999.

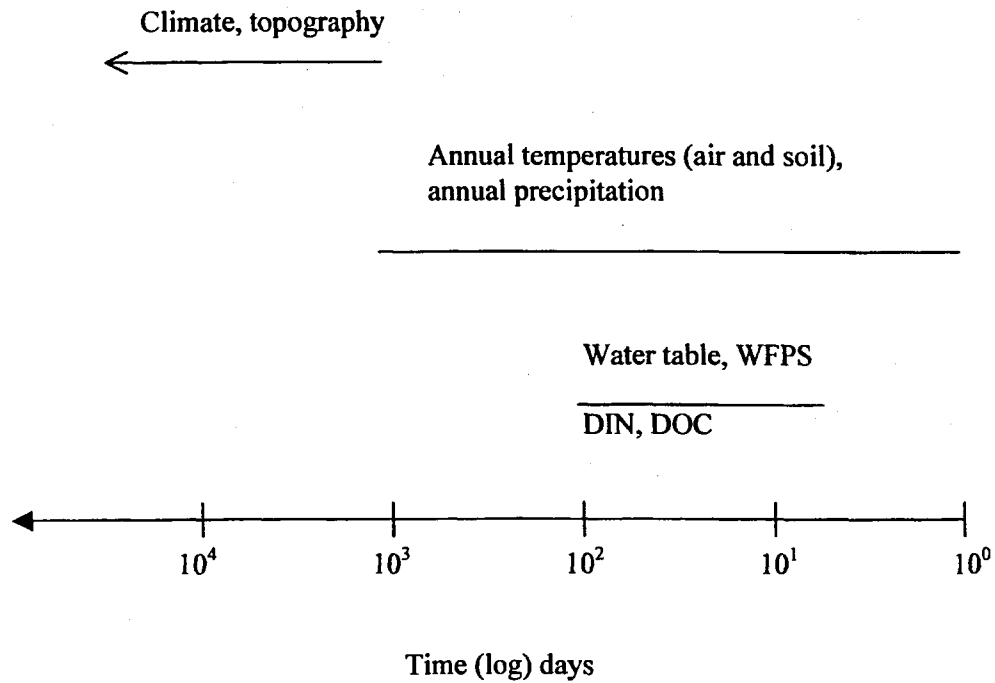
Studies such as Mogge *et al.* (1999), Flessa *et al.* (1995) or Papen and Butterbach-Bahl (1999) showed peaks of N<sub>2</sub>O emissions during freeze-thaw periods. Brumme *et al.* (1999) also showed that freeze-thaw influences N<sub>2</sub>O fluxes but only at one of the sites studied. The effect of temperature on N<sub>2</sub>O emission can be counteracted by its stimulating effect on plant growth, thus enhancing the competition for NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>.

More generally, denitrifying organisms can adapt to local temperatures (and possibly other local conditions): Powlson *et al.* (1988) showed that denitrifiers from England and Australia denitrified at the same rate when at local optimum temperatures of 10 and 20°C, respectively.

## NET EFFECT

The response of N<sub>2</sub>O emissions to factors such as soil moisture, rainfall, N deposition, N fertilisation, carbon source, crop type or temperature is very variable and depends on

the interactions of these factors with each other. A way to better estimate and predict N<sub>2</sub>O emissions in different European ecosystems might be to use emission functions developed from empirical models that use broad controlling factors such as land-use and climate. Fig. 1.2 shows a clear difference between land-uses such as forestry or agriculture. However, these differences in N<sub>2</sub>O emission rates do not necessarily mean that the ecosystems considered differ in their emission factors. They could all have a 1% emission factor and still have very different N<sub>2</sub>O emission rates and receive very different N inputs (atmospheric or fertilisers). An interactive multilayered model in which the controls would be activated by thresholds is shown in Fig. 1.8. These operate over different time scales. For instance, hydrology and mean annual soil temperature are long-term site attributes that are regulated by the regional climate, topography, etc. These establish the overall potential of the site for N<sub>2</sub>O fluxes.



**Figure 1.8:** Major controls on denitrification and nitrification, and approximate time scale of change of importance to N<sub>2</sub>O fluxes.

Threshold values of dissolved inorganic N and DOC and WFPS are then required for actual denitrification or nitrification. The thresholds suggested here by the different studies taken into consideration are 60% of soil moisture (Fig 1.3) and 6 - 10°C for air temperature (Fig 1.7). Figure 1.6 suggests a threshold of 100 t ha<sup>-1</sup> for the organic upper horizon, but more data are required to confirm this value. Variation in these threshold values over a seasonal to weekly time scale will affect the amount of N<sub>2</sub>O released over a given season. Changes in these values may not immediately affect N<sub>2</sub>O fluxes since they may operate by changing competitive relationships among different populations of micro-organisms. Shifts in these relationships may take place after a time lag. A change in soil temperature, however, may immediately affect N<sub>2</sub>O fluxes as it operates on the biochemical scale. If any of the controlling factors is below the threshold, N<sub>2</sub>O flux will not occur. This concept is similar to that developed by Skiba

and Smith (2000) for agricultural systems, and by Ulrich (1994) and Brumme *et al.* (1999) for forest ecosystems. Skiba and Smith (2000) looked at the influence of mineral N, N deposition, land use management, temperature and soil water content on N<sub>2</sub>O emissions from agricultural and natural soils and observed inter-annual variations due to variations in rainfall, timing and intensity and the contribution of N<sub>2</sub>O by indirect sources i.e. ploughing, winter-time emissions or excessive emissions from forest soils in high N deposition areas. They also observed that rainfall and water filled pore space were important controls on variations of N<sub>2</sub>O emissions suggesting that they should be included in the budget equation in order to alleviate uncertainties, ideally in multilayered models rather than simple emission factors. In the study by Brumme *et al.* (1999), a hierarchy of controls on N<sub>2</sub>O emission in forest ecosystems was emphasised. On the basis of process hierarchies in forest ecosystems (Ulrich, 1994), Brumme *et al.* (1999) further developed the conceptual model of the 'hole-in-the-pipe' model of Firestone and Davidson (1989) and Davidson (1991). Ecosystems processes are hierarchically structured according to timescale, ranging from seconds/minutes on the biochemical process level up to centuries on the succession or management level. Long-term control by state variables constrains site and temporal variation in N<sub>2</sub>O emission while short-term controls tend to force the system in another state. Our study focussed on the process level and examined the influence of short-term and long-term controls on such processes. It aimed at finding general relationships between controls and N<sub>2</sub>O emissions.



### 1.3.3 Studies of N<sub>2</sub>O fluxes and/or denitrification in relation to soil moisture, soil temperature, soil nitrate content and soil organic C content

Only five studies have measured N<sub>2</sub>O fluxes and/or denitrification at the same time as all the major factors (soil moisture, soil temperature, soil nitrate and soil organic C) influencing N<sub>2</sub>O emissions. Of these five studies, the first two were by Mogge *et al.* (1998, 1999) who investigated denitrification N-losses and nitrous oxide emissions from forest soils and agricultural soils in the Bornhöved Lake region (northern Germany) over 12 months. The third study, by Clément *et al.* (2002), looked at the seasonal dynamics of denitrification in three riparian wetlands with different vegetation cover in Brittany (France) over 2 years. The fourth study, by Davidson and Swank (1986), reported an investigation of the environmental parameters regulating gaseous nitrogen losses via denitrification at the U.S. Department of Agriculture Forest Service Coweeta Hydrologic Laboratory throughout a 10-month period. The fifth study, by Clayton *et al.* (1994), measured nitrous oxide emissions from a fertilised grassland in central Scotland (UK) regularly for 3 weeks.

Mogge *et al.* (1998) presented the results from *in situ* measurements of denitrification N-losses and nitrous oxide emissions, and the major controls of such emissions at a beech and an alder site. They showed that, in the alder forest soil, temperature accounted for 63% and 44% of the variability of N<sub>2</sub>O emissions and denitrification N-losses, respectively (Mogge *et al.*, 1998). At the seasonal scale highest emissions were observed in summer indicating a positive correlation between soil temperature and gaseous N-emissions. Soil moisture increased during precipitation events from July to September and during December when emission maxima were recorded for both N<sub>2</sub>O emissions and denitrification N-losses in both soils. However, a clear pattern between the precipitation and its influence on the deposition of nitrogen compounds and gaseous N-emissions could not be found. Mogge *et al.* (1998) concluded that sufficient

water as well as amounts of nitrate and organic C generally raises gaseous N-emissions as a result of the microbial turnover.

The second study by Mogge *et al.* (1999) reported an investigation of N<sub>2</sub>O emissions and denitrification N-losses from agricultural soils in the same region of northern Germany. The experiment was carried out in two agricultural fields and one fertilised grassland. They found that, as for forest soils (Mogge *et al.*, 1998), increases in temperature increased gaseous N-losses from agricultural soils. However, they also found that gaseous N-emissions peaked during December after thawing of the soils at all sites. This is consistent with results from other field experiments (Flessa *et al.*, 1995; Kaiser and Heinemeyer, 1996). In comparison to soil temperature, soil moisture had little influence on gaseous N-losses at the experimental scale but was found to be more important at the landscape scale in the Bornhöved Lake Region. Denitrification was limited by nitrate availability at the site receiving less fertiliser. In contrast, gaseous N-emissions were unlimited by nitrogen at the site receiving the greatest amount of nitrogen fertiliser. The content of water-soluble organic-C compounds was highest in the grassland. In the arable soils temporal changes of this variable did not predict gaseous N-losses from the soils. However, peaks of water-soluble organic-C compounds sometimes coincided with high denitrification N-losses.

Clément *et al.* (2002) investigated the seasonal patterns of denitrification rates along the topohydrosequence formed at the upland-wetland interface in three riparian wetlands. The factors limiting denitrification were investigated. They tested the effects of various treatments (i.e., anaerobiosis, anaerobiosis+nitrate, anaerobiosis+carbon as glucose, anaerobiosis+nitrate+carbon). Clément *et al.* (2002) found a significantly higher *in situ* denitrification activity at each of their sites in the upper horizon which corresponds to the organic-rich layer. In the study, the term *in situ* denitrification refers to measurements in unamended soil cores. The results showed that denitrification limiting factors vary seasonally. This seasonal variation along the topohydrosequence of the type of factors limiting denitrification reinforces the fact that this upland-wetland

interface fluctuates seasonally as a function of the ground-water table and most probably the nitrate input from the upslope catchment. These two parameters (i.e., anaerobiosis related to ground-water level fluctuation and nitrate supply) represent the main triggers of denitrification in an environment rich in C. They also found that, in the upper part of the topohydrosequence, close to the upland area, the most important limiting factor for denitrification was the lack of anaerobic conditions. In the lower zones, the denitrification-limiting factor gradually shifted from anaerobiosis to nitrate supply.

Davidson and Swank (1986) reported the results from a study of gaseous N losses from disturbed and reference forested watersheds by *in situ* N<sub>2</sub>O diffusion measurements and laboratory incubations throughout a 10-month period. They showed that soil temperature, percent base saturation (BS), and water filled pore space (WFPS) accounted for 43% of the variation in *in situ* N<sub>2</sub>O diffusion rates. Temperature reflected seasonal variations whereas BS reflected the site variation. They also found that rates of N<sub>2</sub>O diffusion increased as soil moisture increased. Their investigation of denitrification showed that WFPS and redox potential (Eh) accounted for 71% of the variation in denitrification N<sub>2</sub>O and 50% of denitrification N<sub>2</sub>. In this study, Davidson and Swank (1986) also looked at the effects of precipitation on N<sub>2</sub>O diffusion and denitrification. The rates of N<sub>2</sub>O diffusion increased dramatically immediately after precipitation and laboratory estimates of denitrification N<sub>2</sub>O were also higher for soils sampled immediately after precipitation

The last study considered is a short-term experiment by Clayton *et al.* (1994) who measured N<sub>2</sub>O emissions from fertilised grassland regularly for three weeks. They observed the greatest N<sub>2</sub>O fluxes five days after fertilisation and these same fluxes fell to one sixth of their maxima within three weeks. Their investigation showed that wet conditions favoured N<sub>2</sub>O losses through denitrification, mainly from the uppermost 5 cm of soil. They also showed in a regression model of the flux from an ungrazed area,

including the pre- to post-fertilisation transition, that air temperature, recent rainfall, and  $\text{NO}_3^-$ -N could account for 52% of the temporal variability.

These studies confirmed that  $\text{N}_2\text{O}$  fluxes as well as denitrification rates are controlled by few factors such as soil moisture, soil temperature or air temperature, soil nitrate content and soil organic carbon content. However, there is a need to determine which of these factors prevails and what relationship exists between fluxes and such prevailing control.

## **1.4 Role of riparian ecosystems**

Stream riparian zones form an important transition between land and freshwater systems (Gregory *et al.*, 1991), with a significant potential to reduce diffuse pollution, especially  $\text{NO}_3$ ,  $\text{PO}_4$  and pesticides, from agriculture and other human activities. They are complex environments that are spatially heterogeneous in both a horizontal and vertical dimension with respect to hydrology, sediment characteristics, and biogeochemical processes (Hill, 1996). From a water quality perspective, the riparian zone can be divided into two interfaces (Triska *et al.*, 1993). The first one is at the upland interface where materials enter the riparian zone and are transported towards the stream. The second interface, referred to as the hyporheic zone (Triska *et al.*, 1989), is a subsurface zone adjacent to the stream channel where stream water and groundwater are mixing.

Removal of  $\text{NO}_3$  pollution in riparian ecosystems was first studied by Gilliam *et al.* (1974) and Gambrell *et al.* (1975). High concentrations of nitrate in shallow groundwater percolating from row crop fields were found to decline rapidly before reaching the stream channels. Almost all riparian researchers (e.g., Jacobs and Gilliam, 1985 ; Peterjohn and Correll, 1984 ; Lowrance *et al.*, 1984 ; Haycock, 1991) who have looked

at  $\text{NO}_3^-$  have observed that a large percentage of the nitrate in subsurface flows moving toward the streams was removed from the water as it passed through the riparian areas. The only report of failure of a riparian buffer to reduce nitrate was by James *et al.* (1990) in Maryland and this was due to the vegetation growing on that particular riparian buffer (leguminous trees increasing  $\text{NO}_3^-$  in groundwater). Most researchers agree that the primary mechanisms of  $\text{NO}_3^-$  removal in riparian zones are denitrification and plant uptake (Gilliam, 1994).

Riparian zones also have important qualities in terms of the control of erosion and thus prevention of sediment pollution which strongly affects the geomorphology of the stream banks and the water chemistry downstream. The dense root system stabilizes the bank and traps sediment by slowing water runoff from the surrounding area.

## **1.5 Integrated Nitrogen in CAtchments (INCA) model**

### **1.5.1 Introduction**

Nitrous oxide emissions vary widely. Results from European studies (Section 1.3) show that  $\text{N}_2\text{O}$  emissions are not strongly correlated to mean annual precipitation whereas soil moisture levels are a major control, interacting with secondary controls such as N deposition, fertiliser use, carbon source and soil temperature. Nitrous oxide emissions will occur when these controlling factors are not limiting, i.e. above a threshold. Furthermore, the complexity of these interactions makes prediction of  $\text{N}_2\text{O}$  emissions and simple relationships between fluxes and factors difficult to obtain. In order to derive more reliable estimates of  $\text{N}_2\text{O}$  emission, interactive multilayered models are needed which describe N dynamics and  $\text{N}_2\text{O}$  emissions as function of climate and land use.

While these models are lacking, the use of emission factors such as those proposed by the IPCC (1997) or Brown *et al.* (2001) offer the simplest way to estimate N<sub>2</sub>O emissions. However, as Brown *et al.* (2001) show, the IPCC default values are gross approximations. Those wanting more precise estimates must resort to site-specific measurements or dynamic models such as DNDC (Li *et al.*, 1992, 1996), SUNDIAL (Smith *et al.*, 1996) or INCA-N (Whitehead *et al.*, 1998).

DNDC (Denitrification-Decomposition) is a rain-event driven, process oriented simulation model for the evolution of N<sub>2</sub>O, CO<sub>2</sub>, and dinitrogen (N<sub>2</sub>) from agricultural soils (Li *et al.*, 1992). The model consists of three sub-models: thermal-hydraulic, decomposition, and denitrification. Basic climate data drive the model to produce dynamic soil temperature and moisture profiles, and shifts of aerobic-anaerobic conditions. Additional input data include soil texture and biochemical properties as well as agricultural practices. Between rainfall events the decomposition of organic matter and other oxidation reactions (including nitrification) dominate, and the levels of total organic carbon, soluble carbon, and nitrate change continuously. During rainfall events, denitrification dominates and produces N<sub>2</sub>O and N<sub>2</sub>. Daily emissions of N<sub>2</sub>O and N<sub>2</sub> are computed during each rainfall event and cumulative emissions of the gases are determined by including nitrification N<sub>2</sub>O emissions as well.

SUNDIAL (SimUlation of Nitrogen Dynamics In Arable Land) is a PC-based version of the Rothamsted Nitrogen Turnover Model (Smith *et al.*, 1996) which is a complex and dynamic management model. It comprises a menu-driven system that allows agricultural advisers to enter details (soil, weather, fertiliser, organic manure and crop data) of a particular field or farm and simulate N turnover. The processes involved are described by a set of parameterized zero and first-order equations. The facilities in SUNDIAL for displaying the various outputs in graphical form make it particularly useful for examining the impact of different management strategies on the N cycle in arable agriculture.

However, such models are constrained to the N problem in agriculture. Considering the diverse nature of the N problem, an integrated management approach is required (Langan *et al.*, 1997) to assess the likely impacts of land management, N deposition and climate change on European river N concentrations and loads.

### 1.5.2 INCA model overview

INCA (Whitehead *et al.*, 1998a, b) is a model linking hydrological behaviour, the microbiological processes that control N transformation and multiple sources of N inputs to catchments. The model simulates flow pathways and tracks fluxes of both nitrate-N and ammonium-N in the land phase and riverine phase. The dynamic nature of the model means that day-to-day variations in flow, N fluxes and concentrations can be investigated following a change in N inputs such as atmospheric deposition, sewage discharges or fertiliser application. There are five components to modelling nitrogen in catchments using INCA.

1. A *GIS interface* which defines sub-catchment boundaries and calculates the area of six land use classes in each sub-catchment.
2. The *Nitrogen Input Model* which calculates the total N inputs from all sources to each sub-catchment, scaling dry deposition and fertiliser application according to land use.
3. The *Hydrological Model* which models the flow of effective rainfall in the reactive and groundwater zones of the catchment and within the river itself. This component of the model drives N fluxes through the catchment. It consists of three parts. Firstly, the MORECS soil moisture and evaporation accounting model (Meteorological Office, 1981) is used to convert daily rainfall data into an 'effective' rainfall time series. 'Effective' rainfall means the water that penetrates the soil surface after allowing for interception and evapotranspiration

losses. The second component of the hydrological model simulates the effect of land surface or topography on flow. In developing INCA, a semi-distributed approach was adopted so that the dynamics of each sub-drainage basin can be characterised and incorporated into the overall system model. The third component of the hydrological model is the river flow model. This is based on mass balance of flow and uses a multi-reach description of the river system (Whitehead *et al.*, 1979, 1981, 1997). Within each reach, flow variation is determined by a non-linear reservoir model.

4. The *Catchment Nitrogen Process Model* which simulates N transformations in the soil and groundwater of the catchment. This component of the model includes plant uptake and microbial processes such as mineralisation, nitrification, denitrification etc. INCA's Catchment Nitrogen Process Model uses a generalised set of equations with parameter sets specifically derived for the six different land classes. By modifying these parameters, N fluxes from each of the transformations for a given land use can be calibrated against experimental and field data available in the literature. Certain processes such as plant uptake will vary according to land use in terms of both the rate of uptake and the seasonal pattern of uptake. Microbial N transformations within the soil are temperature and moisture dependent, both of which can vary according to land use. Within INCA, land use can be viewed as an approximate surrogate for soil type for a number of characteristics which influence N transformations, although the effect of more complex soil properties such as % C and N, and C:N ratio are not accounted for in the model. The Catchment Nitrogen Process Model takes the output from the Nitrogen Input Model (INCA-GIS interface), which gives wet and dry ammonium-N and nitrate-N inputs from atmospheric deposition, together with fixation and fertiliser input to each land use sub-catchment. An initial condition is also required for surface and groundwater concentrations. In a sense, the initial conditions represent the state of the catchment and land use



at that point in time. The user therefore needs to either measure groundwater and soil water nitrogen as input initial condition or estimate these from model calibration against field data.

5. The *River Nitrogen Process Model* which simulates dilution and in-river N transformations and losses such as nitrification and denitrification. Net N output from each sub-catchment (component model 4) provides the N flux into the corresponding river reach and input to the River Nitrogen Process Model. This component receives ammonium-N and nitrate-N inputs from the Catchment Nitrogen Process Model (soil reactive zone and groundwater zone sources) and N inputs from the direct discharge of sewage effluent and urban runoff. The key processes operating in each reach of the river are nitrification and denitrification. The reach mass balance must include the upstream nitrate-N and ammonium-N in addition to the catchment and effluent inputs. Water flows from the sub-catchments to drain into the river reaches and the river equations are solved to maintain a mass balance along the river. Additional inputs from sewage effluents or industrial discharges can be incorporated into the reach structure mass balance and the user specifies these inputs.

In more recent years INCA has been used as part of a programme sponsored by the European Commission under the EVK1-1999-00011 contract number studying nitrogen dynamics across Europe, from Northern Scandinavia to the Mediterranean and from continental Germany to the maritime United Kingdom. This project started in April 1999 and aims to apply the INCA model across Europe to investigate these N dynamics for key ecosystems. Its application to a wide range of ecosystems and river catchments is designed to make it a tool for assessing the N dynamics in both the plant/soil system and in river networks. Also, the streamwater N concentration and load changes induced by changes in land management, pollutant inputs and the climate are predicted and the results used to infer the likely impacts at the pan-European scale (Wade *et al.*, 2002).

The specific objectives of the project are as follows:

1. to establish hydrological and water quality databases for a range of key European ecosystems;
2. to apply the process-based dynamic model, INCA, to these selected catchments across Europe;
3. to establish N budgets in the catchments and compute fluxes of N on a daily, seasonal and annual basis both for the plant/soil system and in stream;
4. to modify the model process equations as necessary to develop a generic model applicable to a wide range of ecosystems and catchments;
5. to use the model to assess the impacts of land management, atmospheric deposition and climatic change in the catchments selected;
6. to compute fluxes of nitrous oxide gas release from catchments, riparian zones, wetlands and stream beds;
7. to investigate model parameter and structural uncertainty and scale-up from the plot to the large (c. 4000km<sup>2</sup>) catchment scale;
8. to add an economic component to the INCA project to assess the costs of a range of N controls in agriculturally intensive catchments within Europe;
9. to create an easy to use Windows version of INCA with high quality graphics for management and scientific use;
10. to make INCA available to the European Environment Agency, National Environment Agencies, the Water Industry and other interested parties.

### 1.5.3 Purpose and Role of N<sub>2</sub>O within the INCA model

Over the past thirty years, the problem of N contamination of both terrestrial and freshwater environments has shifted from a local pollution issue to a regional one (Neal *et al.*, 2002). This N pollution issue threatens to spread to a continental scale if no measures are taken to reduce excessive NO<sub>3</sub><sup>-</sup> inputs (Heathwaite *et al.*, 1993). The concerns already existing at the European scale not only relate to terrestrial and aquatic environments but also to climate change problems where N<sub>2</sub>O is an important greenhouse gas produced by soils. It is therefore important that the INCA model simulates the release of N<sub>2</sub>O from the nitrification and denitrification processes in order to assess the impact of land management changes on the emissions of N<sub>2</sub>O and possible consequences in terms of global warming. In INCA, both processes depend on the soil moisture deficit. Currently, INCA estimates denitrification using the following equation:

$$\text{Denitrification} = - C_1 S_1 (x_5 / (V_r + x_{11})) 10^6 \quad (1)$$

where  $C_1$  is a constant and represents the denitrification rate in  $\text{m day}^{-1}$ ,  $x_5$  is the ammonium stored in the soil in  $\text{kg N km}^{-2}$ ,  $x_{11}$  is the soil water volume in  $\text{m}^3 \text{ km}^{-2}$ .  $S_1$  is the soil moisture factor (no unit) and  $V_r$  is the soil retention volume in  $\text{m}^3 \text{ km}^{-2}$ . They are calculated as follow:

$$S_1 = (SMD_{\max} - U_5) / SMD_{\max} \quad (2)$$

where  $U_5$  is the input soil moisture deficit time series (mm) and  $SMD_{\max}$  is the maximum soil moisture deficit (mm).

$$V_r = V_{r, \max} - U_5 \cdot 1000 \quad (3)$$

where  $V_{r, \max}$  is the maximum soil retention volume ( $\text{m}^3 \text{ km}^{-2}$ ), equals:  $d * p * 10^6$  (4)

where  $d$  is the soil depth (m),  $p$  is the soil retention porosity (no units) and the factor of

$10^6$  is included to maintain the dimensions in Eqn. (4). In INCA, denitrification increases with soil wetness (Groffman *et al.*, 1996).

## **1.6 Aims/Objectives and layout of the thesis**

The literature review presented above suggests that nitrous oxide emissions are mainly controlled by soil moisture and soil temperature interacting with secondary controls such as carbon source, fertiliser use and N deposition. The complexity of these interactions along with spatial and temporal variability of  $N_2O$  emissions make prediction of such emissions and simple relationships between those and controls difficult to obtain.

Many studies have shown that large quantities of  $NO_3^-$  in soil water are effectively removed in the riparian zone of streams draining agricultural land before entering into downstream surface waters. The beneficial effects of nitrate removal are, however, countered by the detrimental effects of nitrous oxide. Since long-term field measurements of  $N_2O$  emission are rare it is not clear how significant riparian zones are to the global  $N_2O$  budget. In addition, the conditions under which the proportion of total nitrogen released by denitrification shifts from  $N_2O$  to  $N_2$  (inert atmospheric nitrogen) are unclear.

The purpose of this study, therefore, was to establish which of the factors (i.e. soil temperature, soil moisture, soil nitrate- and ammonium-N, soil organic carbon, rainfall) known to control the mitigation of nitrogen pollution to a river system by release of gaseous N, especially  $N_2O$ , has the major effect by measuring fluxes from two riparian ecosystems in the UK along with those environmental factors. Another question raised by the study was how significant are riparian zones to  $N_2O$  release from agricultural

land. Also, the riparian ecosystems studied differed by their nature. One is representative of the common riparian ecosystem found in the UK with a steep drop-off from the field to the stream while the other has a very smooth slope from field to stream. This difference is expected to have an influence on the resulting mitigation of N pollution and  $\text{N}_2\text{O}$  release by each ecosystem and this hypothesis was tested. Denitrification was the other focus of this study, with the particular aim of determining which conditions favour the proportion of total nitrogen released by denitrification to shift from  $\text{N}_2\text{O}$  to  $\text{N}_2$  in the ecosystems studied. The study eventually aimed to empirically relate the  $\text{N}_2\text{O}$  fluxes and the denitrification rates to the main environmental controls. The resulting relationship between denitrification and main environmental control would then be used in the INCA model to better estimate denitrification in riparian ecosystems.

**Chapter 2** details the principal methods used during the thesis, and gives a description of the field site and the different experimental plots. It also presents a description of a complementary field experiment using acetylene to measure denitrification and laboratory experiment.

**Chapter 3** presents the results obtained during the first year for the measurement of  $\text{N}_2\text{O}$  fluxes from two riparian experimental sites differing in their hydrology and the nature of the agricultural fields they are draining. The study aims to determine the factors controlling the  $\text{N}_2\text{O}$  emissions at these sites, whether the hydrological differences have any influence on emissions and controlling factors, and the most appropriate way for modelling these fluxes. It is mainly in the form of a scientific journal article and has been published in the journal *Water, Air and Soil Pollution*.

**Chapter 4** details the investigation of the processes both within a riparian ecosystem and in the laboratory on soil collected at the two main experimental plots and explores the conditions under which the end product of the denitrification process will shift from  $\text{N}_2\text{O}$  to  $\text{N}_2$ , as well as the potential for denitrification in the soils studied. This chapter also includes a discussion of the implications of such results on  $\text{N}_2\text{O}$  emissions.

In **Chapter 5** a version of the INCA model, developed for riparian ecosystems, is calibrated to the Bedford Ouse river system using data from the year 2001 to 2003 in order to obtain a set of parameters that are then used to apply the model to the experimental sites. The results from this application are then compared to the findings from chapter 3 and 4.

**Chapter 6** presents a general discussion of the combined findings presented in the different investigations and summarises the main conclusions of the study.

## **Chapter Two**

### **Materials and methods**

#### **2.1 Introduction**

In this chapter, the main methods used to investigate the effect of soil temperature, soil moisture, soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in solution and rainfall on  $\text{N}_2\text{O}$  fluxes from two different riparian ecosystems in south-central England are described. A detailed description of the experimental sites is also provided. In addition, the methods available for the measurement of  $\text{N}_2\text{O}$  fluxes are discussed.

#### **2.2 Nitrous Oxide flux measurements**

##### *2.2.1 Introduction*

Methods available for the measurement of trace gas emissions (in this case  $\text{N}_2\text{O}$ ) from soils fall in two main categories: enclosure, or chamber methods and micrometeorological techniques. Both techniques have their advantages depending on the information required from the investigation. Chamber techniques are commonly used to measure trace gas fluxes ( $\text{CH}_4$ ,  $\text{N}_2\text{O}$ ,  $\text{NO}$ , etc.) and have a number of

advantages over micrometeorological methods (Smith *et al.*, 1994). They greatly reduce the labour requirement for field sampling and analysis and make much more feasible both long-term measurements and intensive short-term investigations required for process-related studies. Also, they make possible measurements at remote sites over longer periods, or more frequently, than is possible by means of field campaigns or sampling expeditions. They are relatively inexpensive to make and can be used in a wide variety of environments. Another advantage is that they are suitable for determination of small fluxes, which may be below the detection limits of micrometeorological methods. The main reason why chambers were used in this study is that they are a good method for the measurement of fluxes from systems where land use practices involve small fields with different crops and fertilizer rates, plots with different treatments, and so on. However, chambers are not without problems which are discussed in section 2.2.2.

Micrometeorological methods offer a different set of advantages over chamber techniques. The most important one is that fluxes may be measured over larger areas and they have been shown to integrate average small-scale spatial variability that may be encountered by chamber methods. A full review of micrometeorological methods can be found in *Fowler and Duyzer (1989)*.

### *2.2.2 Problems associated with chamber methods and their minimisation*

Two basic chamber types have been used in the field. The first, known as closed or 'static' chambers, involves the calculation of flux by periodically taking samples from within a defined chamber 'headspace' and then measuring the rate of change in gas concentration during the period of linear concentration change (Fig 2.2; section 2.3.2). The second chamber type is the open or 'dynamic' chamber. In this method, a



continuous flow of air from the atmosphere is allowed into the chamber via an air inlet, over an area of soil defined by the chamber. The air then leaves the chamber via an outlet and the flux is calculated from the change in concentration between the outlet and the inlet to the chamber, the chamber area and the flow rate. This method allows a better approximation of conditions experienced naturally. However, measurement error can be easily introduced through changes in pressure within the chamber leading to possible underestimation or overestimation of the natural rate of gas flux. Also, the measurement system requires electricity and air pumps or batteries which are bulky and heavy equipments as well as quite expensive. This made the method unsuitable for the main field study reported here (Chapter 3). A detailed review of chamber methods can be found in *Mosier (1989)*.

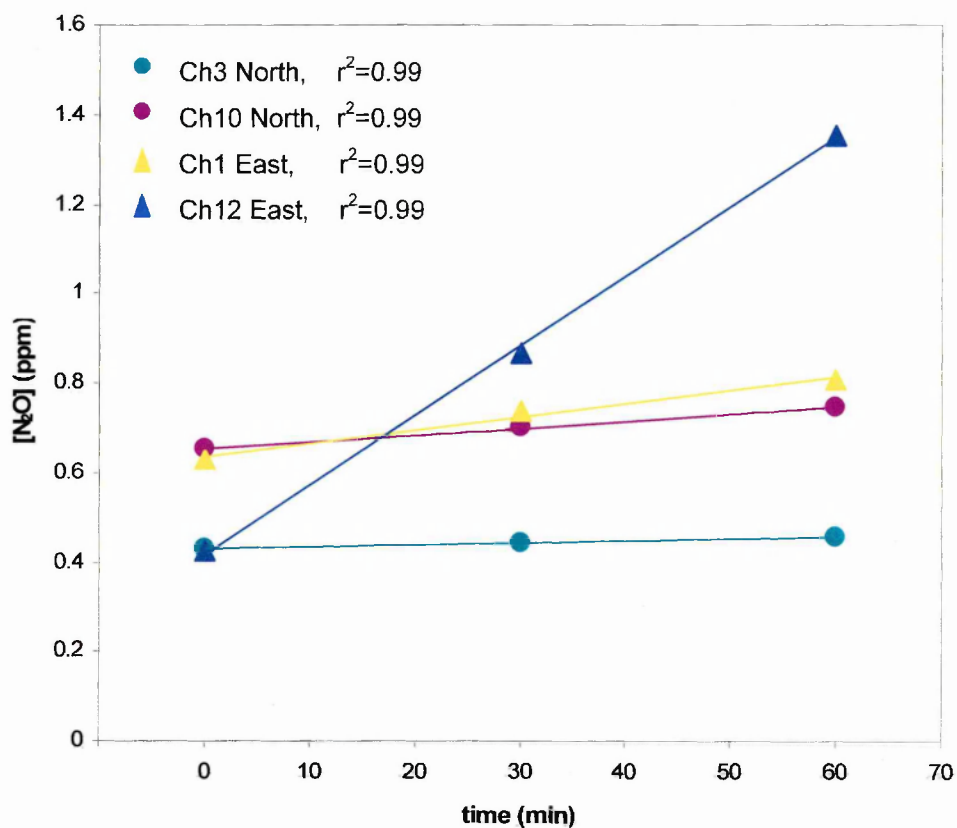
#### *Site Disturbance*

Site disturbance can happen when inserting chambers into soils and may affect the processes or transport mechanisms that determine rates of emission for the gas being investigated. This can occur through a variety of ways. Compaction of the soil when chambers are inserted can have the temporary effect of increasing fluxes. In the study at Chicheley (section 2.3 and Chapter 3), this potential source of error was minimised by using a cutting knife to facilitate insertion of collars into the soil, which could then be repeatedly sampled through the addition of a lid, which defined a chamber headspace.

#### *Concentration Effects*

An important mechanism for the emission of  $N_2O$  from soils to the atmosphere is diffusion, which depends on a concentration gradient between the point of production and the point of emission (i.e. the atmosphere). A build up of  $N_2O$  in the headspace can limit further emission via diffusion (Mosier and Heinemeyer, 1985). This was minimised in the Chicheley study by keeping closure times to a minimum. Preliminary

tests with chambers placed at the different sites showed that this effect was insignificant for closure times of up to an hour, as  $\text{N}_2\text{O}$  concentration increase within the chamber was linear over this time (Fig 2.1).



**Figure 2.1:** Linear increase in chamber headspace  $\text{N}_2\text{O}$  concentrations for four chambers at the Chicheley sites.

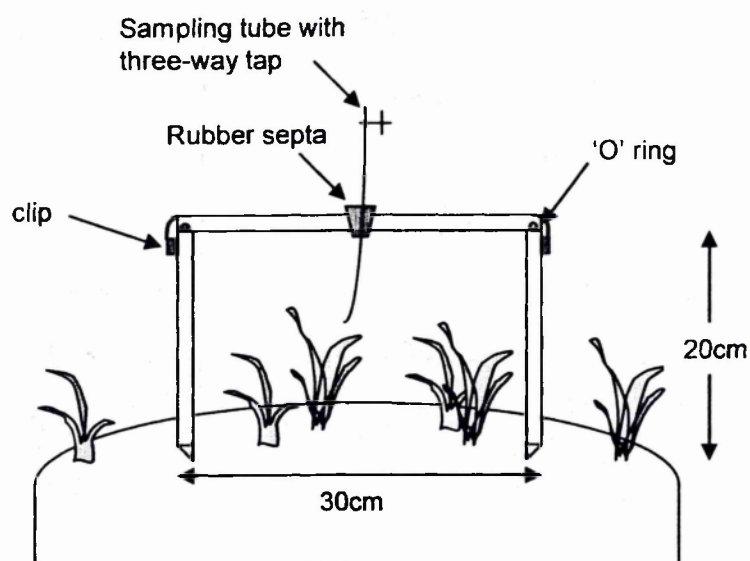
### *Temperature Effects*

Microbial processes responsible for N<sub>2</sub>O emissions are strongly influenced by temperature. Since the chamber acts as a greenhouse, there is a possibility that changes in temperature may increase fluxes. However, it has been shown that in practice it takes far longer for soils to increase in temperature than air in the chamber volume and that the increase in soil temperature over a one hour enclosure period is not significant (MacDonald, 1998). In the experiments reported in this thesis all samples were taken within one hour of a chamber being closed. The chamber lids were clear but the chambers were made of opaque material which minimised the radiative effect.

### *Pressure Changes*

Closed chamber methods can also prevent fluctuations in atmospheric pressure from affecting fluxes within the enclosure. Such fluctuations may stimulate emissions by increasing soil air movement, and so excluding enclosed air masses from such natural variability may result in underestimating real flux rates (Mosier, 1989). This problem may be alleviated by inserting a vent; however, no such mechanism was included in this study since the time intervals of the measurements were too short for such effects to occur.

The main purpose of the experiments reported here was to evaluate the difference in N<sub>2</sub>O fluxes at two different riparian ecosystems and determine the relationships existing between the environmental factors controlling N<sub>2</sub>O fluxes and the fluxes themselves. It is therefore likely that the methods employed in this study (sections 2.3 and 2.4) were appropriate means of meeting this objective.

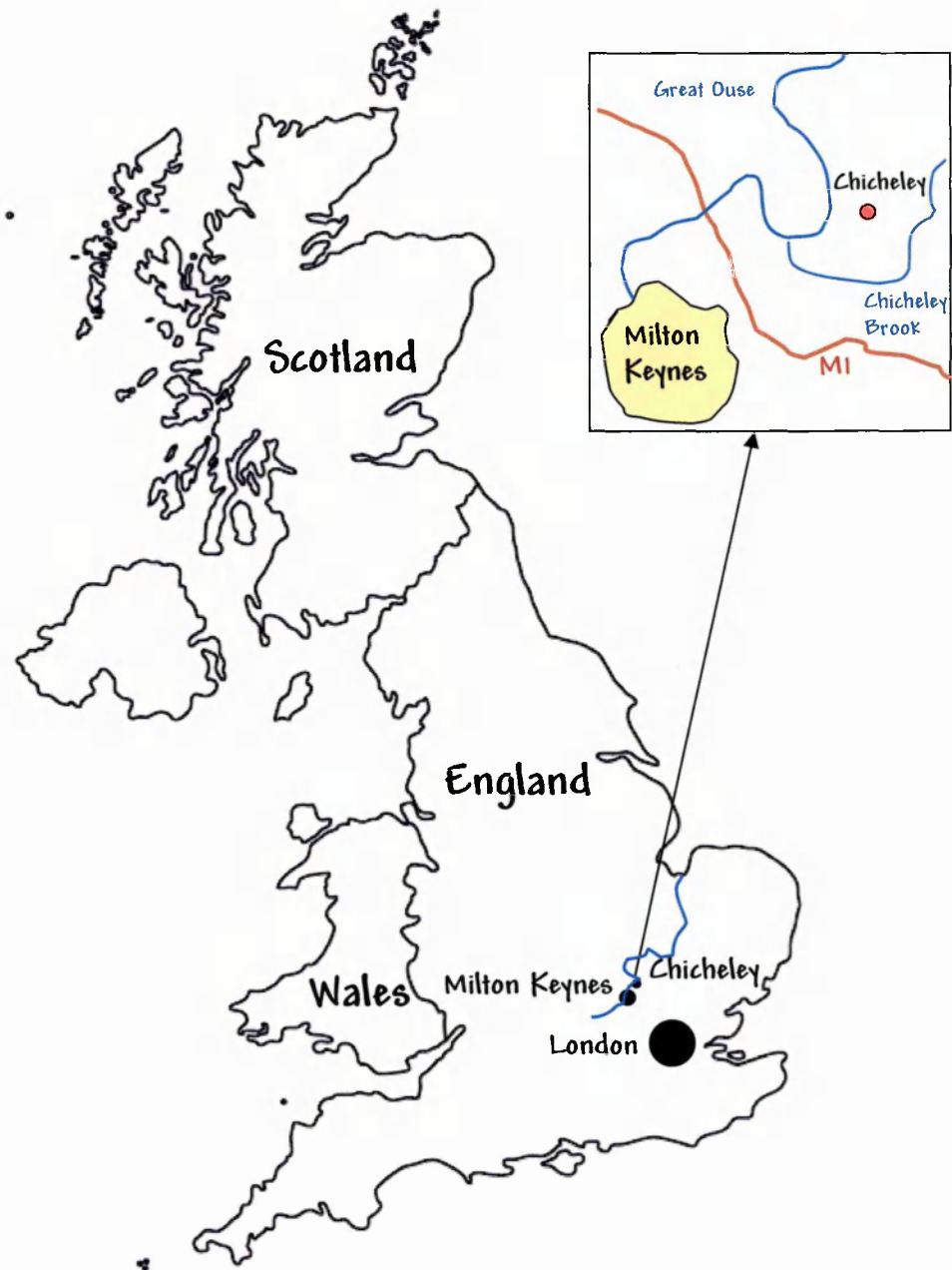


**Figure 2.2:** Chamber methods used at Chicheley experimental sites.

## 2.3 Field Study of N<sub>2</sub>O fluxes

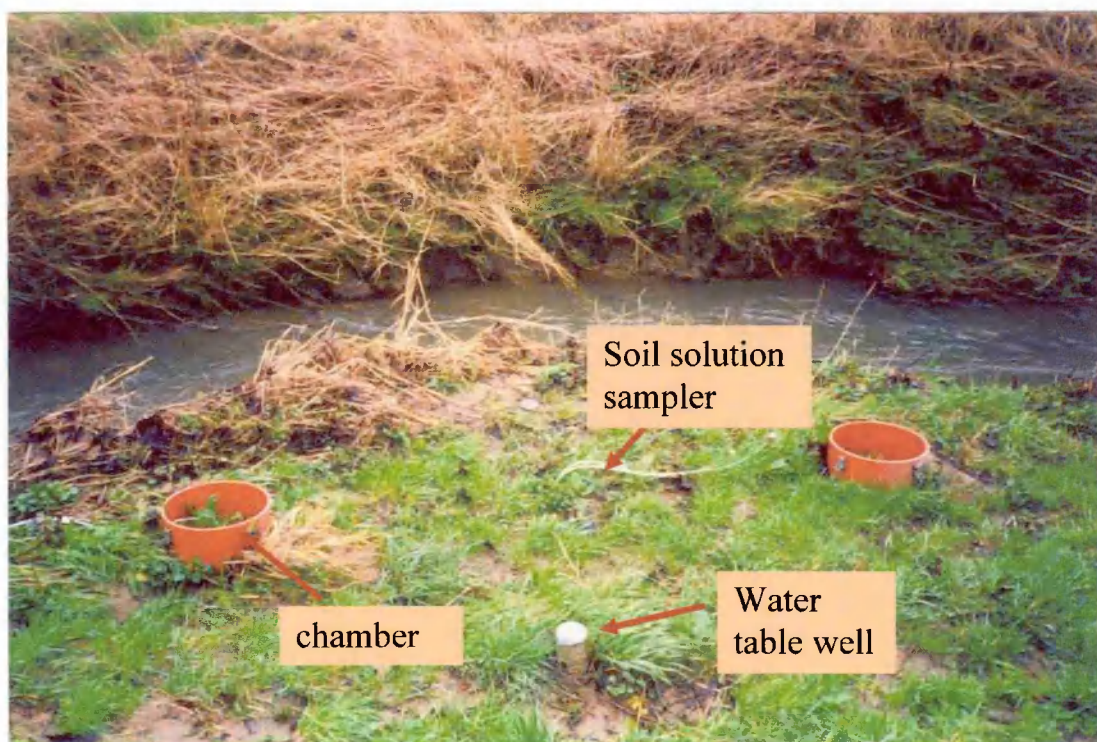
### 2.3.1 Site descriptions

For the field study of N<sub>2</sub>O fluxes two main experimental plots were chosen within the Great Ouse river catchment, located near the village of Chicheley, north east of Milton Keynes, United Kingdom (SP904 440GB; Fig 2.3).



**Figure 2.3:** Sites location. Blue line on the main map represents the river Great Ouse.

$N_2O$  fluxes were measured using static chambers placed within each experimental plot for the duration of the measurement period (Fig 2.2). A detailed description of the experimental methods can be found in Chapter 3 (section 3.3). Ceramic cups were used to withdraw some soil solution for analysis for nitrogen content at the two experimental plots, and wells were installed for monitoring water-table levels (Fig 2.4).



**Figure 2.4:** Experimental site at Chicheley.

### 2.3.2 Sample collection

The headspace volume was defined by placing a clear Perspex lid on top of the chamber. Each lid was fitted with a rubber septum pierced to allow a 2.5 mm O.D. tube to be inserted in order to sample the headspace gas with a syringe. Repeated sampling was made over an hour as described in Chapter 3, section 3.3. Prior to the start of the experiment, the Exetainers (10mL-glass vials) were tested for leakage by filling them with 100 ppm  $N_2O$  standard. The  $N_2O$  concentration of the Exetainers was then analysed via gas chromatography (see section 2.3.3) both at time = 0 days and time = 7 days. Results showed that a 6.5% ( $N=3$ ,  $STDEV=0.6$ ) loss in  $N_2O$  concentration occurred over the 7 days. However, the samples were brought back to the laboratory the same day as collected and analysed within 24 hours. Each chamber was sampled weekly to fortnightly between April 2001 and November 2002.

N<sub>2</sub>O fluxes were calculated using the equation

$$\text{Flux (N}_2\text{O)} = \frac{\Delta C \times f \times V}{a \times t}$$

Where  $\Delta C$  is the change in N<sub>2</sub>O concentration (ppm),  $f$  is a concentration to mass conversion function,  $V$  is the volume of the headspace (m<sup>3</sup>),  $A$  is the soil area defined by the chamber (m<sup>2</sup>) and  $t$  is the enclosure time (hours). The flux measurements are reported here in mg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup>.

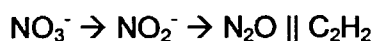
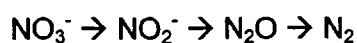
### 2.3.3 Method of analysis

N<sub>2</sub>O concentrations were analysed on an Ai Qualitek GC94 gas chromatograph fitted with an electron capture detector GC ECD (Appendix II) with a 1.23m long Porapak Q (50–80) backflush column and a 1.83m long Porapak Q (50–80) analysis column. On each analysis occasion, the GC was calibrated against a 100 ppm N<sub>2</sub>O in N<sub>2</sub> standard diluted to 10 ppm and 5 ppm with compressed N<sub>2</sub> gas and 1 ppm standard (from Alltech and Supelco Sigma-Aldrich, respectively). The detection limit for the GC was 0.417 ppm N<sub>2</sub>O with a standard deviation of 0.167 ppm.

## 2.4 Acetylene Study

### 2.4.1 Introduction

Over 30 years ago, Federova *et al.* (1973) discovered that acetylene inhibited the reduction of N<sub>2</sub>O to N<sub>2</sub> in the denitrification process. This discovery formed the basis for the development of the acetylene inhibition method (Balderston *et al.*, 1976; Yoshinari & Knowles, 1976).



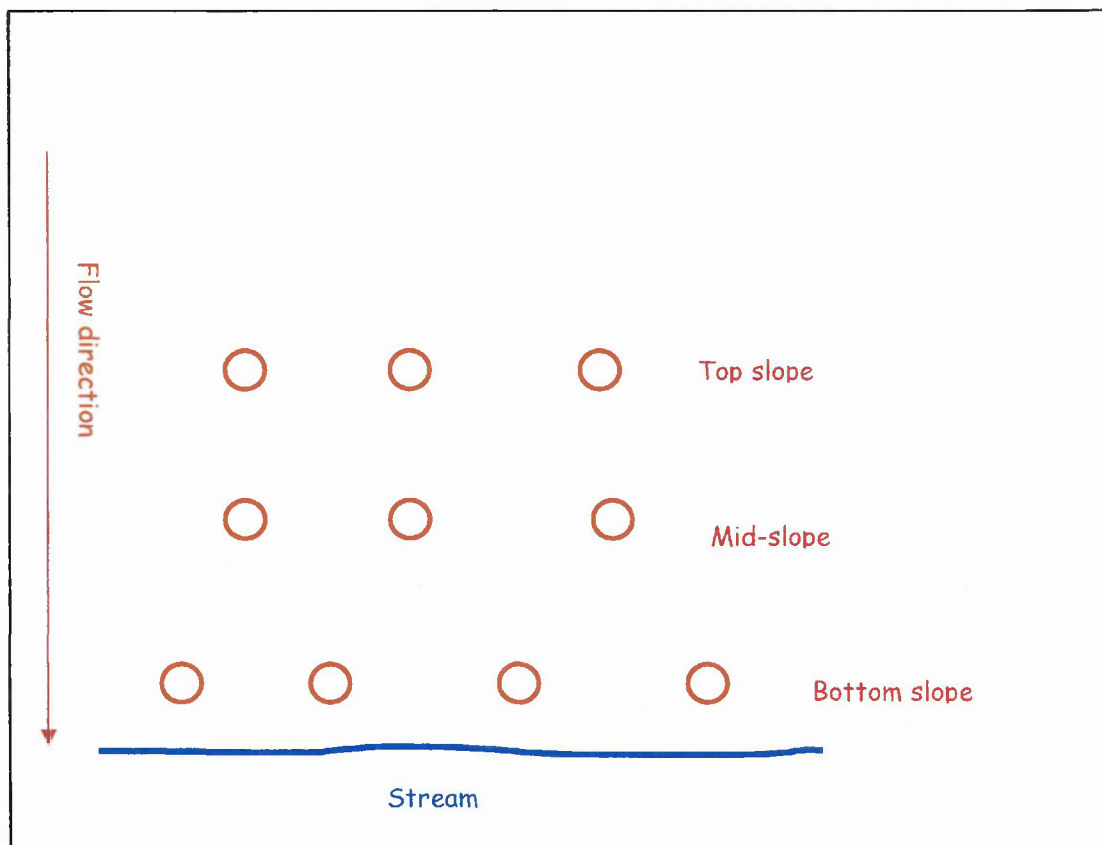
Previously it was difficult to measure  $N_2$  production during denitrification because of the high background concentration of the gas in the atmosphere (78%). By inhibiting the last step in the denitrification process with acetylene, low denitrification rates can be measured in ambient atmosphere by measuring  $N_2O$  emissions, given the low natural background concentration of  $N_2O$  of about 310 ppb (Duxbury, 1986; Klemmedtsson *et al.*, 1990).

#### 2.4.2 Site description

The experimental plot is within the Great Ouse river catchment (UK). It is located near the town of Chicheley, north east of the city of Milton Keynes (52.3° N, 0.7° W). The site is a riparian ecosystem situated in an active farm and drains an agricultural field into a small stream: the Chicheley Brook. The plot is characterised by a gradual slope (17%) down to the stream and a long runoff from the field. The soil is from the Fladbury Series, a grey clayey pelo-alluvial gley with >50% clay in the plough layer (0-25 cm), and the soil pH (in  $H_2O$ , 1:2.5) averages around 7.6.

Ten 'static' chambers were installed at the site. The chambers were made of 30 cm diameter PVC rings inserted 5 cm deep in the topsoil at three levels above the stream surface. The number of replicate chambers was 4 and 3 at the lower level, nearer to the stream, and the other two levels, respectively (Fig. 2.5).





**Figure 2.5:** Diagram of experimental design of the Chicheley acetylene experiment. The experimental plot is 6m x 7m, the brown circles represent the flux chambers.

### 2.4.3 Acetylene method

In order to determine denitrification rates an initial measurement of the  $N_2O$  emission was carried out. Samples were collected according to the same protocol as described in section 2.3.2. All chambers were then vented and lids replaced. Acetylene was applied directly by replacing 10% of the headspace of the chambers by acetylene gas. The gas was left to diffuse for two to three hours and chambers were then opened and vented once again. Denitrification was then measured following the same sample collection protocol as in section 2.3.2 but with samples taken every fifteen minutes over half an hour.

#### 2.4.4 Problems associated with acetylene method and their minimisation

The acetylene inhibition method can be used with the closed chamber technique in the field. However, the acetylene method should not be used for long-term measurements of denitrification since prolonged exposure of the soil to acetylene may lead to accelerated acetylene utilisation or incomplete blockage of  $\text{N}_2\text{O}$  reduction to  $\text{N}_2$  (Mosier and Klemmedtsson, 1994). In this study, this problem was addressed by measuring denitrification only once a month, leaving enough time for the soil to recover from the acetylene application before the following measurement. Another concern when using the acetylene inhibition technique in the field is the effect of soil texture and water content on gas diffusion (Letey *et al.*, 1980). In flooded soils and probably in wet clayey soils, the movement of the gases produced in the soil to the atmosphere above is controlled by water. Since a gas diffuses about 10 000 times more slowly in water than in air, the time required for a gas to move from its production site in the soil to the atmosphere may depend upon soil water content. To counteract this possible problem, the acetylene was left to diffuse for longer when soils were waterlogged (three and a half hours). When using the acetylene method one must be aware that there might be a risk of underestimating the rate of denitrification since acetylene also inhibits oxidation of ammonium ( $\text{NH}_4^+$ ) by nitrifying microorganisms (Hynes and Knowles, 1978; Bremner and Blackmer, 1979). However, this effect is thought not to be of high importance (Ryden and Dawson, 1982; Colbourn *et al.*, 1984; Terry *et al.*, 1986) especially in agricultural soils where nitrate is not limiting (Tiedje *et al.*, 1989). Inhibition of nitrification which is coupled to denitrification can cause incorrect estimations of denitrification if nitrate concentrations are limiting (Malone *et al.*, 1998) which was not the case at the study site. Nitrification can also produce  $\text{N}_2\text{O}$ . However, alternation of denitrification and nitrification in riparian zones will only occur if the oxidation/reduction potential (Eh) is not low enough to allow denitrification at significant rates (Duff and

Triska, 1990; Jones *et al.*, 1994). This may be short-term, as associated with periods between rainfall events, or longer-term due to extended drought (Correll, 1997). In addition, in this study, the soil ammonium content was always negligible so that the nitrification contribution to N<sub>2</sub>O production was considered of secondary importance.

## **2.5 Denitrifying Enzyme Activity (DEA) Laboratory study**

### **2.5.1 Introduction**

The objective of the denitrification enzyme assay is to estimate the concentration of functional denitrifying enzymes in a soil sample at the time of sampling. The measurement is based on the principle that when conditions for an enzyme-catalysed reaction are optimised, the reaction rate is proportional to the enzyme concentration. In this assay, denitrifying conditions are optimised by saturating the system with substrate carbon and NO<sub>3</sub><sup>-</sup> and removal of O<sub>2</sub>. Chloramphenicol is added to prevent protein synthesis during the assay. However, chloramphenicol may affect on the denitrification process (Smith and Tiedje, 1979; Dendooven *et al.*, 1994) and should only be used if the period of DEA measurement is short. Acetylene is added to a concentration of approximately 10% of headspace gas volume. Acetylene must be purified to remove acetone, which can lead to serious overestimation of denitrifying activity since it is readily utilised by denitrifying microorganisms for reduction of NO<sub>3</sub><sup>-</sup> (Gross and Bremner, 1992). Adequate replication (three or four) is required. The N<sub>2</sub>O produced is measured by gas chromatography. Because the assay aims to determine the potential of a soil for denitrification at the time of sampling and this depends on the soil conditions at that time, measurements have to be made on fresh soil as soon as possible after it has been sampled.

Prior to carrying out the DEA assay on a regular basis, a trial experiment was done on soils sampled from different soil depths, in order to establish the exact location of maximum denitrification activity at the study sites. The soil depths considered were 0 to 10 cm, 10 to 20 cm and 20 cm to the water table. The results showed that the high denitrification activity generally occurred at 0 – 10 cm depth. The DEA was thereafter carried out on fresh soils sampled at this depth.

### ***2.5.2 Experimental protocol***

The experimental protocol for the denitrifying enzyme activity method employed in this study is presented in Appendix III.

### ***2.5.3 Calculations***

The calculations for determination of the potential denitrification rate are provided in Appendix III.

## **2.6 Other Analyses**

### ***2.6.1 Soil physical analyses***

#### ***Bulk Density measurement***

Soil bulk density is defined as the ratio of the mass of oven-dried solids to the bulk volume of the soil at some specified soil water content, usually that at sampling (*Blake and Hartge, 1986*). Bulk density is an extremely useful parameter, as it is required to calculate porosity when particle density is known, to convert weights to volumes, and to estimate the mass of soil volumes too large to weigh. It is also required to convert mass-based determinations to a volume basis which are often of more interest. Bulk

density is a dynamic soil property used to characterise soil structure. It is also an important parameter for understanding hydraulic and biogeochemical processes, and it is a good indicator of soil texture and organic matter content which affects the soil temperature and pH. The procedure involves driving a cylindrical (known volume: V) core into the soil, excavating it carefully and removing the soil core sampled into a pre-weighed aluminium pan (W1). The soil core and aluminium pan are then placed in an oven set to 105°C over night. After drying and cooling in a desiccator, the weight of aluminium pan plus dry soil is recorded as W2 (g). The bulk density (BD, in g cm<sup>-3</sup>) is then calculated as follow:

$$BD = (W2 - W1)/V$$

#### *Soil moisture*

##### **Gravimetric water content**

In the DEA experiment, water content was measured by gravimetric analysis as described in Appendix IV and water content calculated as percent water filled pore space (WFPS) also described in Appendix IV.

##### **Volumetric water content**

In the main field study, volumetric water content was measured using *time domain reflectometry* (TDR; Dalton, 1992). In TDR, two stainless steel rods are inserted parallel to one another to a given soil depth (10 – 100 cm); an electrical pulse sent through these wave guides generates an electrical response (read with an oscilloscope) that is characteristic of the dielectric constant for a soil at a particular water content. The major drawback of TDR at this time is the high initial cost of equipment. However, its convenience and accuracy make the method extremely attractive.

The TDR approach (Dalton, 1992) allows continuous or intermittent measurement of total soil water content with minimal disturbance. TDR represents the best available method for regular monitoring of soil water content.

Volumetric water content can also be obtained by multiplying gravimetric water by the bulk density (Appendix III). However, this method was not used in this study since it is time consuming in the laboratory and a reasonable number of replicates would be necessary to obtain an accurate determination.

#### *Soil pH*

The soil pH was measured using a standard method where one part of the soil by weight was shaken with 2.5 parts of distilled water by volume and left to equilibrate for 30 minutes (Thomas, 1996; Appendix V).

#### *Particle Size Analysis*

Particle size was measured by hydrometer and classified according to the USDA classification scheme [i.e., sands ( $< 2000 - 50 \mu\text{m}$ ), silts ( $< 50 - 2 \mu\text{m}$ ), and clays ( $< 2 \mu\text{m}$ )]. The hydrometer method allows for non-destructive sampling of suspensions undergoing settling and provides for multiple measurements on the same suspension so that detailed particle-size distributions can be obtained with minimum effort. The hydrometer method outlined is that modified from Gee & Bauder (1986) (Appendix VI).

#### *2.6.2 Dissolved ions*

Major anions (e.g.,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{F}^-$ ,  $\text{SO}_4^{2-}$ ) and major cations (e.g.,  $\text{NH}_4^+$ ,  $\text{Na}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ) in the soil solutions were analysed using a Dionex Ion Chromatograph as described in Appendix VII.

### *2.6.3 Dissolved Inorganic Nitrogen and Dissolved Organic Nitrogen*

$\text{NO}_3^-$  and  $\text{NH}_4\text{-N}$  concentrations in solution (dissolved inorganic nitrogen, DIN) along with total nitrogen (TN) were determined using a Skalar SAN<sup>PLUS</sup> continuous flow analyser. Dissolved organic nitrogen (DON) was then calculated as the difference between TN and DIN.

#### *Total Nitrogen UV digestion*

The automated procedure for the determination of Total N (range 1 to 50 ppm N) is described in Appendix VIII.

#### *Ammonia-N*

The automated procedure for the determination of  $\text{NH}_3$  (range 0.2 to 10 ppm N) is based on the modified Berthelot reaction (Appendix VIII).

#### *Nitrate-N*

The automated procedure for the determination of  $\text{NO}_3^-$  (range 0.2 to 10 ppm N) and nitrite is based on the cadmium reduction method (Appendix VIII).

### *2.6.4 Dissolved Organic Carbon*

Dissolved organic carbon was measured in the soil solution using a total organic carbon analyser Thermalox (Analytical Sciences, Cambridge). The Thermalox's measuring system can detect total carbon (TC), total inorganic carbon (TIC), and non

purgeable organic carbon (NPOC, corresponds to the organic carbon, as all the inorganic carbon has been removed prior to C determination). The procedures for calibration and sample preparation can be found in Appendix IX. Because NPOC is desired in this study, the inorganic carbon must first be driven out of the solution (Appendix IX).

## **2.7 Data Analysis**

### **2.7.1 Statistics**

Conventional statistical packages (Microsoft Excel 2002 and XLStat versions 7.0 and 7.1) were used in this study to evaluate the effects of environmental factors on N<sub>2</sub>O fluxes and model the possible relationships between such factors and the fluxes.

Statistical methods that are specific to individual chapters are discussed where relevant.

### **2.7.2 INCA**

A version of INCA especially developed for riparian ecosystems was calibrated using data from the year 2001 to 2003 for the Bedford Ouse river system. Once the model was calibrated, the parameters obtained from the calibration were used to run an application of the model to our experimental sites and the results obtained from this application were then compared to the field data.



## **Chapter Three**

# **Controls on the emission of nitrous oxide from two agricultural riparian ecosystems**

(A version of this chapter is published in: Machefert S. E., Dise N. B., Goulding K.W.T. and Whitehead P.G., Nitrous oxide emissions from two riparian ecosystems: key controlling variables, Water, Air and Soil Pollution – Focus, 2004, 4, 427-436)

### **3.1 Abstract**

Nitrous oxide ( $\text{N}_2\text{O}$ ) emissions were measured weekly to fortnightly between April 2001 and November 2002 from two riparian ecosystems draining different agricultural fields. The fields differed in the nature of the crop grown and the amount of fertiliser applied. Soil temperature and soil water content were very important controls of  $\text{N}_2\text{O}$  emission rates, with a 'threshold' response at  $8^\circ\text{C}$  and 24% moisture content (by volume), below which  $\text{N}_2\text{O}$  emission was very low.  $\text{N}_2\text{O}$  fluxes were higher at the site that had received the most fertiliser N where flooding happened more frequently, but  $\text{NO}_3^-$  was not a limiting factor at either site. There was also a 'threshold' effect of rainfall, in which major rainfall events ( $\geq 10\text{mm}$ ) triggered a pulse of high  $\text{N}_2\text{O}$  emission if none of the other

environmental factors were limiting. These results suggest the existence of multiple controls on N<sub>2</sub>O emissions operating at a range of spatial and temporal scales. Non-linear relationships, perhaps with a hierarchical structure, are the most appropriate way to model N<sub>2</sub>O emissions from riparian ecosystems.

### 3.2 Introduction

N<sub>2</sub>O is one of the most important anthropogenically-enhanced greenhouse gases, behind CO<sub>2</sub> and methane (CH<sub>4</sub>). It contributes ca 6% to global warming (Denmead, 1991) and is involved in the destruction of stratospheric ozone (Crutzen, 1970). About 70% of the total globally-emitted N<sub>2</sub>O is derived from soils (Bouwman, 1990) and agriculture as a whole (i.e. animal excreta, denitrification of leached nitrate, etc.) contributes ca 81% of the anthropogenic N<sub>2</sub>O emissions (Brown *et al.*, 2001).

Stream riparian zones form an important transition between land and freshwater systems (Gregory *et al.*, 1991), with a significant potential to reduce diffuse pollution, especially nitrate, phosphate and pesticides, from agriculture and other human activities. For example, forested and grass buffer strips can reduce N in subsurface waters by 40-100% and 10-60%, respectively (Osborne and Kovacic, 1993). A principal process that removes nitrate from water moving through riparian zones is denitrification, in which the nitrate is reduced to N<sub>2</sub>O and N<sub>2</sub> (e.g., Burt *et al.*, 1999). Complete reduction to N<sub>2</sub> effectively closes the N cycle and benefits the environment as nitrate is removed from water without release of N<sub>2</sub>O to the atmosphere. Partial reduction to N<sub>2</sub>O, however, swaps one pollutant for another. Riparian areas are known to be "hotspots" of N<sub>2</sub>O production in the landscape, especially when they receive and process large amounts of excess nitrogen from agricultural fields (Groffman *et al.*, 2000).

The amount of N<sub>2</sub>O emitted from riparian zones depends on the physical, chemical and biological attributes of soil, on climate and weather conditions, and on complex interactions among these factors (Teira-Esmatges *et al.*, 1998). Factors known to influence soil N<sub>2</sub>O emission include available N (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>), temperature, soil moisture content, carbon availability (Conrad, 1996), climate (Ambus and Christensen, 1995) and hydrologic flow. Very few studies of N<sub>2</sub>O emission from riparian ecosystems have considered both N<sub>2</sub>O fluxes and all of the major environmental controls measured at regular intervals over a full year, however. The high variability of the fluxes, complex interactions among the controls and lack of understanding of the processes involved make such studies difficult. Models of N<sub>2</sub>O emission exist but there is a need for more integrated approaches to tackle the problem.

This chapter presents the results obtained from measurements of N<sub>2</sub>O fluxes from two riparian ecosystems differing in their hydrology and the nature of the agricultural fields they drain. The study aims to determine the factors controlling the N<sub>2</sub>O emissions at these sites, whether the site hydrological differences have any influence on emissions and controlling factors, and the most appropriate way for modelling these fluxes.

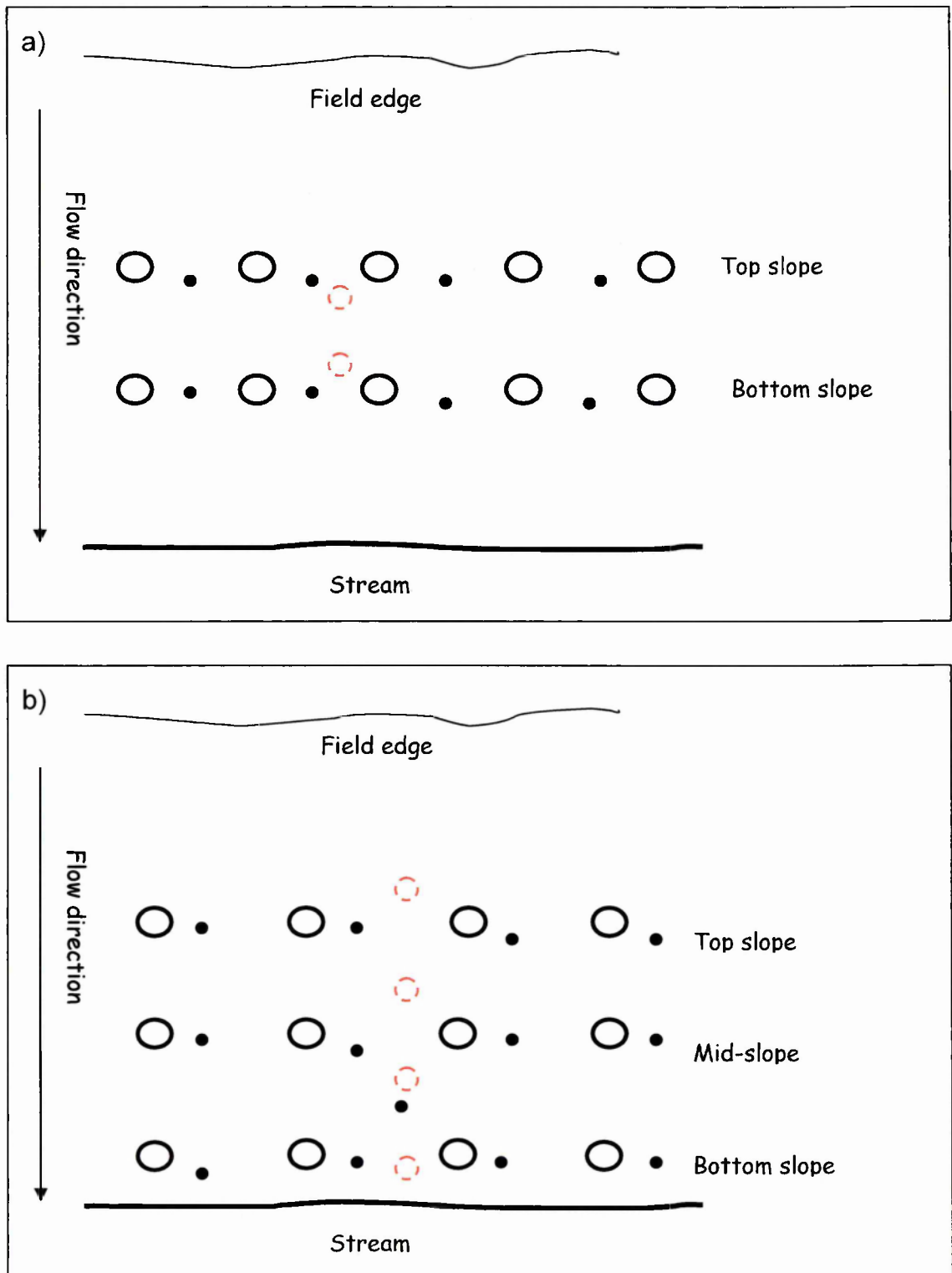
A second overall aim of the study is to evaluate the usefulness of emission factors as a tool for setting pollutant control legislation. Emission factors are a statistical average of the rate at which a pollutant is released to the atmosphere as a result of some activity divided by the rate of that activity (Stern, 1977). In order to provide an estimate of current rates and assess change in N<sub>2</sub>O emissions, one of the obligations of signatory states of the United Nations Framework Convention on Climate Change (UNFCCC) is to establish a national emission inventory that fully reports all anthropogenic sources of greenhouse gases, using comparable methodologies (Brown *et al.*, 2001). To this end, protocols have been developed by the Intergovernmental Panel on Climate Change (IPCC, 1997) which provides a methodology for calculating emissions using defined emission factors. For this purpose, agricultural N<sub>2</sub>O emissions are assumed to be derived from three principal sources (IPCC, 1997):

- direct emissions from soil nitrogen (N), e.g. applied fertilisers in both manures and artificial (chemically fixed N) forms, N deposited by grazing animals, mineralization of crop residues, biological N fixation and cultivation of high organic content soils;
- emissions from animal waste management systems;
- indirect emissions from N lost to the agricultural system, e.g. through leaching, runoff or atmospheric deposition.

### 3.3 Site and methods

Two experimental plots were chosen within the Great Ouse river catchment (UK). They are located near the village of Chicheley, north east of Milton Keynes, and will be referred to as Chicheley North (or CH North) and Chicheley East (or CH East) in the rest of the chapter. Both sites are riparian ecosystems situated in an active farm and drain different agricultural fields into the same small stream: the Chicheley Brook. Chicheley East plot (7.6m x 7.6m) is characterised by a gradual slope (17%) down to the stream and a long runoff from the field. From April to September 2001, it drained an oilseed rape field receiving 302 kgN/ha/yr. Chicheley North plot (2.5m x 19.7m) is representative of the more common riparian ecosystem found in the UK, with a steep drop (40% slope) from the field to the stream. From April to September 2001, it drained a wheat field receiving 226 kgN/ha/yr. From September 2001, both crops were reversed. The fertiliser was applied as urea and Liquid N37 (37 % N w/v, solution fertiliser) on two different occasions at both sites: 06/03/2001 and 12/04/2001. The soils are from the Fladbury Series, a grey clayey pelo-alluvial gley with >50% clay in the plough layer (0-25 cm), and the soil pH (in H<sub>2</sub>O, 1:2.5) averages 7.6.

Twenty-two 'static' chambers were installed *in situ* at the two sites: 10 at Chicheley North and 12 at Chicheley East (Fig 3.1). The chambers were made of 30 cm diameter PVC rings inserted 5 cm deep in the topsoil at two and three levels above the stream surface at Chicheley North and Chicheley East, respectively. The number of replicate chambers at each level was 5 at Chicheley North and 4 at Chicheley East. N<sub>2</sub>O fluxes were determined using the closed chamber technique (Hutchinson and Mosier, 1981). Gas samples (20 mL) were withdrawn from the headspace using a 60 mL syringe immediately after closing the chamber, and 30 minutes and 60 minutes later, and after the atmosphere in the chamber had been mixed by pumping the syringe plunger 6 times. Each sample was injected into an evacuated container (Labco Exetainer, 10 mL). The change in the N<sub>2</sub>O concentration as a function of time gave the flux rate for N<sub>2</sub>O emission or, in the case of a decrease in concentration over time, adsorption by the soil.



**Figure 3.1:** Experimental setup at a) Chicheley North and b) Chicheley East. Black circles represent the chambers, black dots represent the ceramic cup lysimeters and red interrupted circles represent the water-table wells. The plots are 2.5m x 19.7m and 7.6m x 7.6m for Chicheley North and Chicheley East respectively.

After each hour long sampling period, the chambers were left open until the following sampling. Ambient air samples were also taken at each visit. The Exetainers were transported to the laboratory and  $\text{N}_2\text{O}$  concentrations were determined using a gas chromatograph fitted with an electron capture detector and equipped with a PorapakQ, 50-80, 6ft column. The carrier gas ( $\text{N}_2$ ) flow rate was  $58\text{ mL min}^{-1}$ , the detector temperature was  $320^\circ\text{C}$ , the injector and the oven were at  $45^\circ\text{C}$  and  $60^\circ\text{C}$ , respectively. Air temperature using a digital thermometer, soil temperature at 10 cm depth and soil moisture content (% vol. TDR, 6cm probe) were monitored on a weekly to fortnightly basis at each sampling date. Total daily rainfall was obtained from a meteorological station about 6 km south of the sites. Water-table wells were used to monitor the groundwater level.  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations were determined every two weeks in the soil solutions obtained from 8 and 13 ceramic cup (Fairey Industrial Ceramics Limited) lysimeters inserted 35 cm deep around the gas sampling points at Chicheley North and Chicheley East, respectively, using ion chromatography. DON was also measured in the same soil solutions at the same frequency using a Skalar SAN<sup>PLUS</sup> System.

## **3.4 Results and discussion**

### **3.4.1. FLUXES**

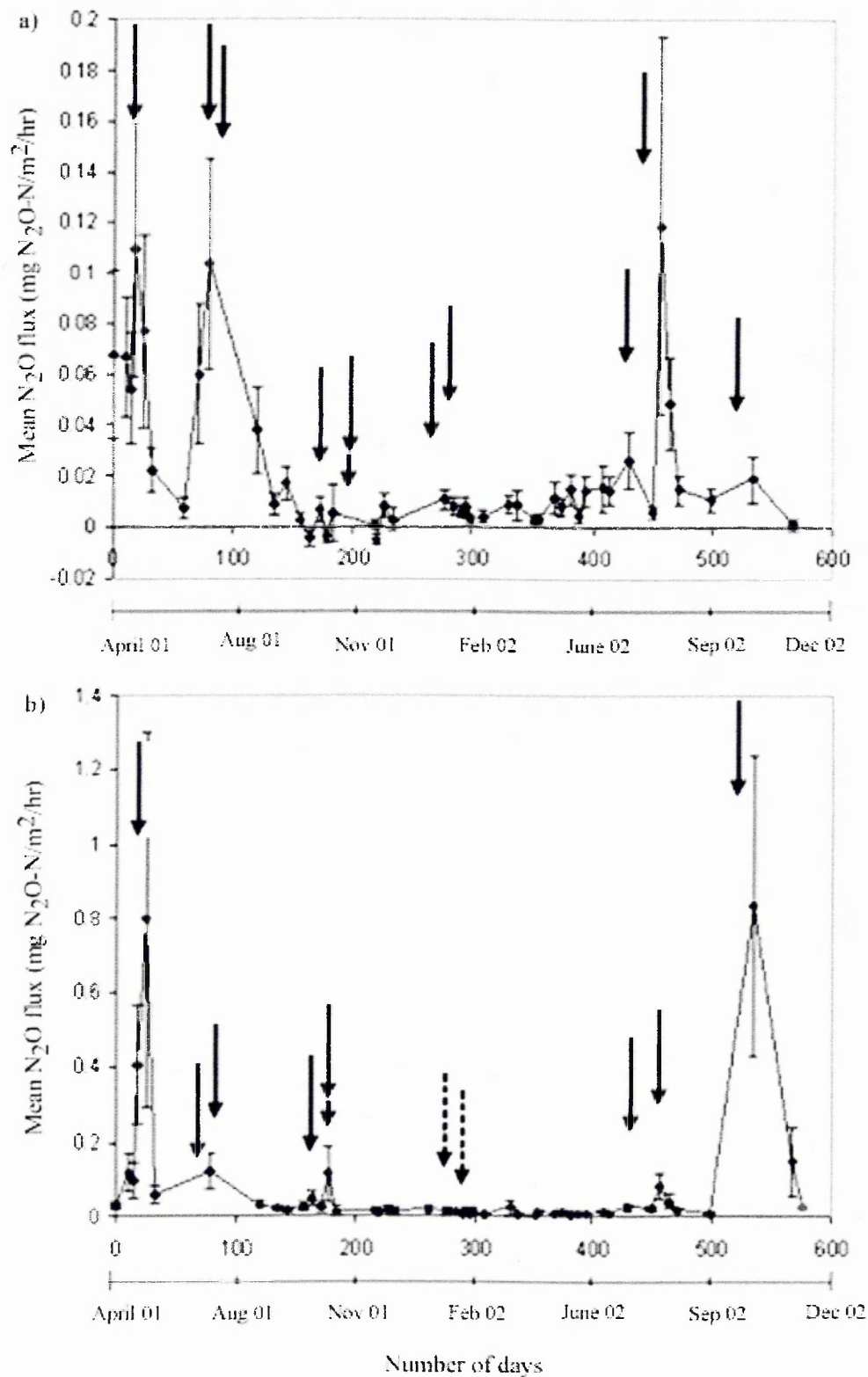
Nitrous oxide fluxes measured throughout the measurement period displayed high temporal and spatial variation (Fig 3.2). Similar high variability has been found for  $\text{N}_2\text{O}$  fluxes from other temperate climate riparian zones (Hanson *et al.*, 1994; Groffman and Tiedje, 1989). Despite this high variability, some general trends emerged. Fluxes were generally higher in spring/summer than in winter, and fluxes at Chicheley East were

generally higher than at Chicheley North. Some small negative fluxes were observed at Chicheley North, which indicates that the soil was able to take up atmospheric N<sub>2</sub>O (Granli and Bockman, 1994).

At Chicheley North, the maximum mean N<sub>2</sub>O flux observed was 0.109 mg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup> (0.026 kg N ha<sup>-1</sup> d<sup>-1</sup>) and the maximum single value was 0.344 mg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup> (0.083 kg N ha<sup>-1</sup> d<sup>-1</sup>). The maximum mean value observed at Chicheley East was 0.798 mg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup> (0.191 kg N ha<sup>-1</sup> d<sup>-1</sup>) and the maximum single value was 4.54 mg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup> (1.09 kg N ha<sup>-1</sup> d<sup>-1</sup>). At both locations, the highest rates of N<sub>2</sub>O emission were observed at the down slope positions where the water table fluctuated closest to the surface, especially at CH East down slope, which was sometimes flooded.

The total N<sub>2</sub>O fluxes at CH East and CH North were 5.502 kg N ha<sup>-1</sup> and 0.625 kg N ha<sup>-1</sup>, corresponding to 1.82 and 0.28 % of the N applied, respectively. These values are within the range of "emission factors" quoted by Brown et al. (2001) for direct emission of N<sub>2</sub>O from soil fertilised by N (0.25 % to 2.25 %). Total fluxes from both riparian ecosystems studied are also characteristic of emissions from N-enriched terrestrial ecosystems (Machefert et al., 2002; Chapter 1).

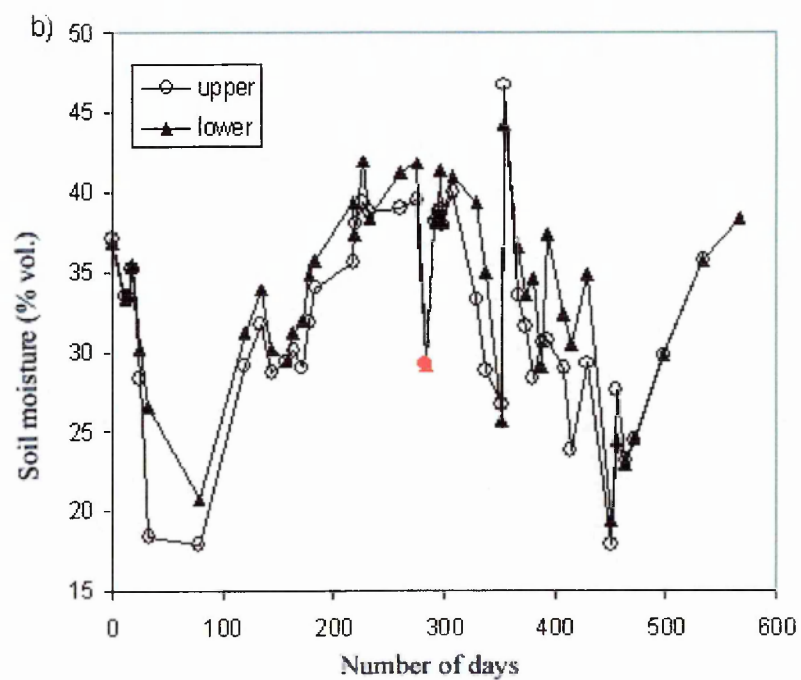
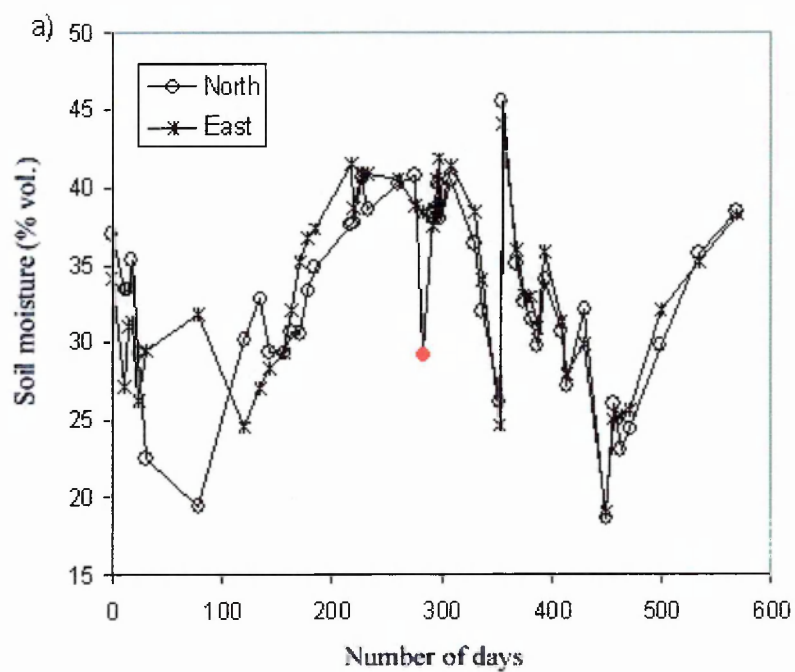


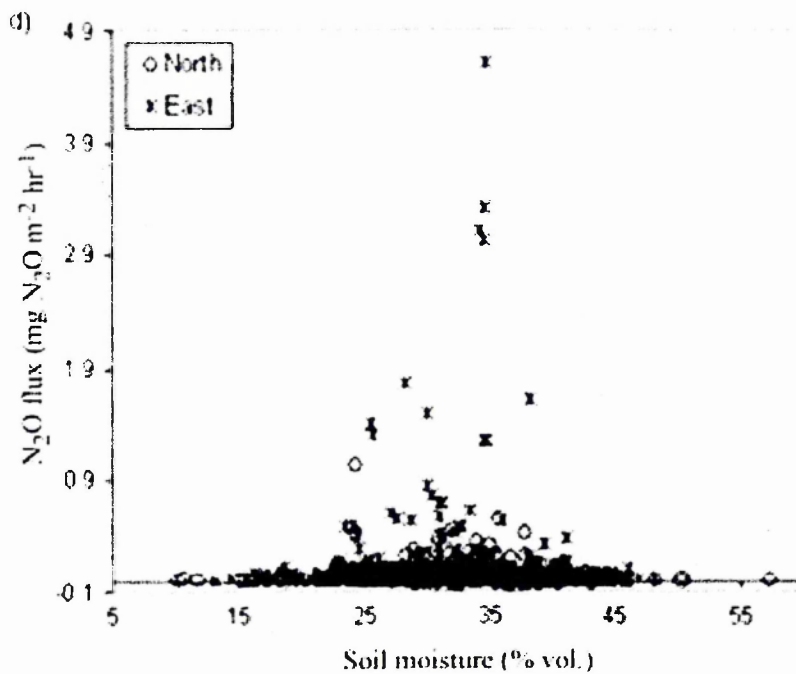
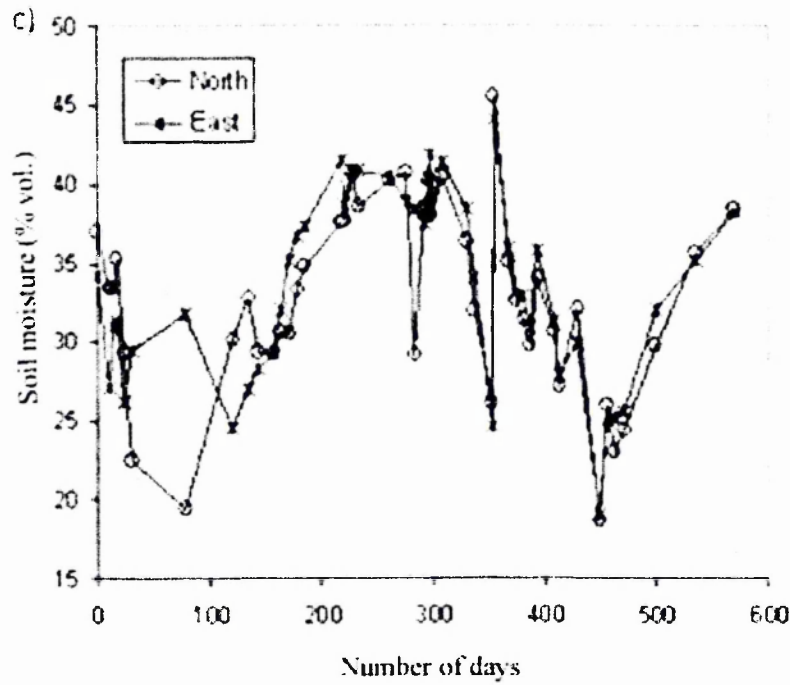


**Figure 3.2:** Mean  $N_2O$  fluxes (with standard errors) and principal rainfall events at (a) Chicheley North and (b) Chicheley East sites. The black arrows represent total daily rainfall events of 10 mm or more when all the other factors are above threshold levels (temperature  $8^\circ C$  and 24 % volumetric water content). The dotted arrows represent total daily rainfall events of 10 mm or more when at least one other factor was limiting. Number of days are from 30/04/2001 (day 0) to 19/11/2002 (day 568). The final point (red) on Chicheley East plot was measured at the acetylene inhibition location.

### 3.4.2. SOIL MOISTURE

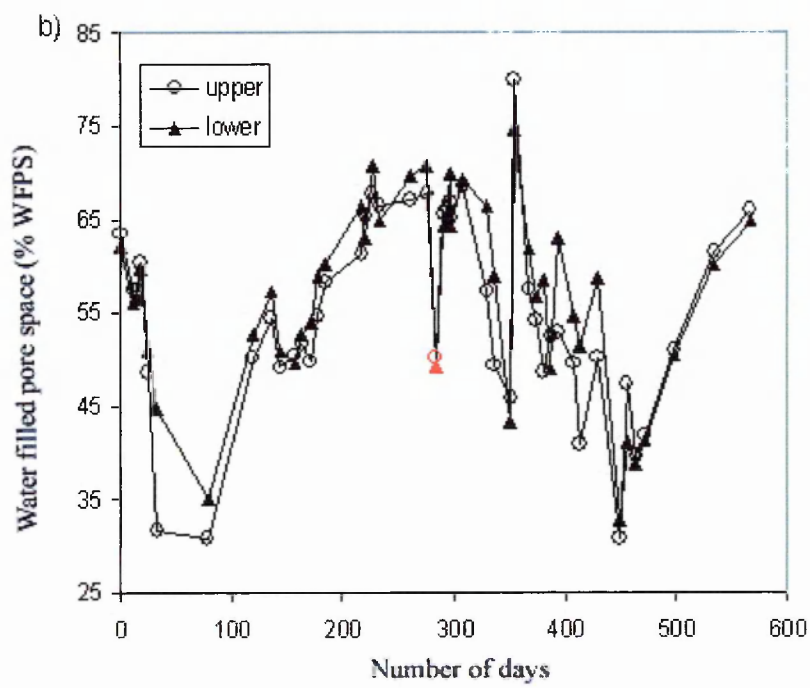
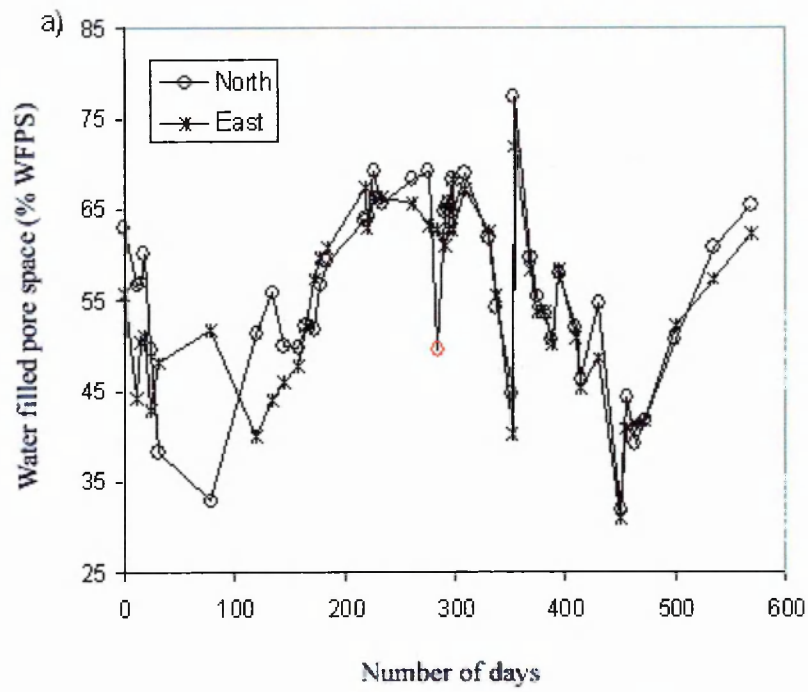
Throughout the measurement period the overall soil moisture increased, as spring 2001 was dry and the rest of the year was relatively wet. Overall, mean soil moisture as well as WFPS were not significantly different from one plot to the other (Fig 3.3a and 3.4a), but there were important differences in the pattern of soil moisture between the sites. Chicheley North generally showed higher soil moisture and water filled pore space (WFPS) at the lower slope location (Fig 3.3b and Fig 3.4b), especially when some time had elapsed after a major rain event. This is expected since we observed a higher water table at the lowest slope locations. At Chicheley East the upper slope location usually had the highest soil moisture and the lower slope location often had the lowest (Fig 3.3c). This result is unexpected at the Chicheley East experimental plot since a clear difference of water table was observed. A possible explanation could come from differences in soil texture. Frequent flooding at the lowest slope location resulted in sediment deposition. The soil here should be sandier than the upslope locations and drainage would be quicker. Recalculating the Chicheley East data as WFPS (which should partly account for the effects of texture) shows some changes with respect to soil moisture (Fig 3.4c), although in general the upper slope still has higher WFPS as well as higher soil moisture. A particle size analysis of the soils at the different levels of the slope showed that the bottom of the slope is sandier than the soil from the top of the slope. Percentages of sand for the upper, intermediate and lower slope locations are 39.6%, 37.4% and 57.2%, respectively. The analysis showed that both upper and intermediate locations were silt loam soils and the lower location was sandy loam. This suggests that weekly measurements may be insufficient to capture the dynamics of rapid water level changes at the lowest locations on the slope. It also suggests that in some cases (e.g. for soils) WFPS may not capture all of the effects of texture on soil hydrology and anaerobicity.

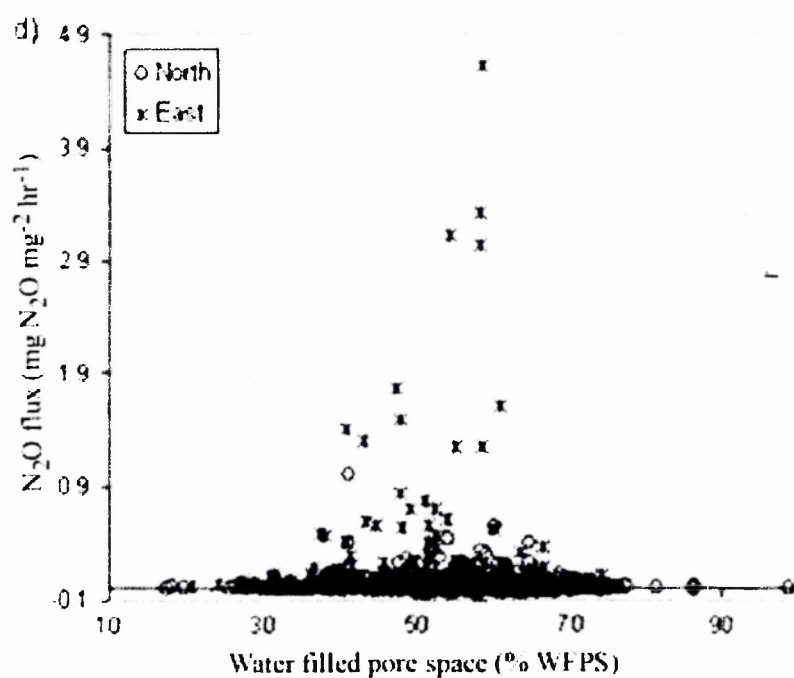
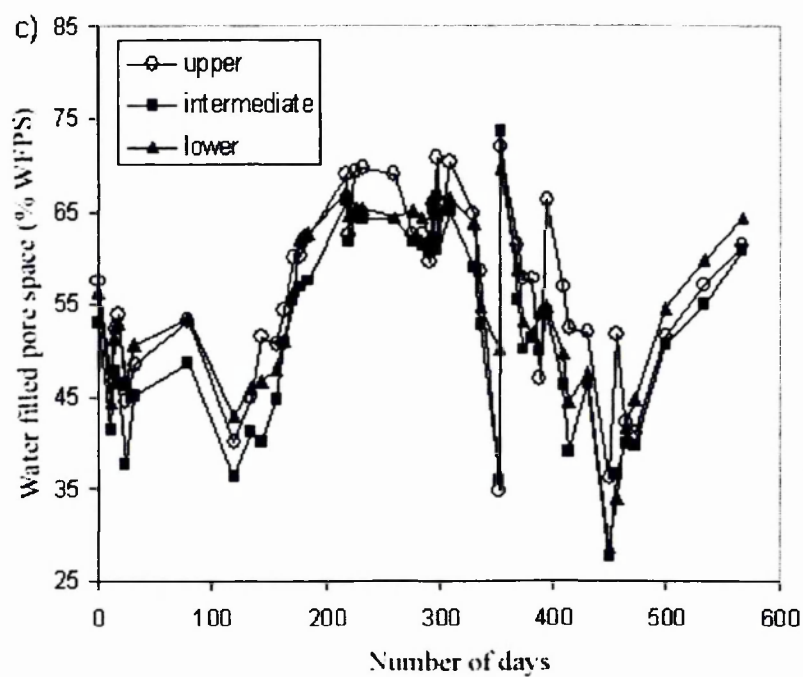




**Figure 3.3:** Mean soil moisture at a) both experimental plots, b) the upper and lower locations of the slope at Chicheley North, c) the upper, intermediate and lower locations of the slope at Chicheley East. d) Relationship between  $N_2O$  flux at both experimental plots and the soil moisture. Red dots in plots a) and b) represent measurements where there might have been a problem with the equipment.

No linear relationships were found between the N<sub>2</sub>O fluxes and volumetric soil moisture or WFPS (Fig 3.3d and Fig 3.4d). However, a clear non-linear pattern was observed. The individual N<sub>2</sub>O fluxes for each of the 22 chambers on each sampling date plotted against the soil moisture (% vol.) showed very low fluxes at low soil moisture contents followed by an increase of fluxes when soil moisture reached 24% vol (Fig 3.3d). The maximum flux was observed at about 35% vol. of the soil moisture (Fig 3.4d). Then, N<sub>2</sub>O fluxes started to decrease. Although our 24% threshold was not statistically significant due to too few low moisture data points, a similar threshold (25% vol.) was found by Granli and Bockman (1994). The decrease in fluxes observed when soil moisture reached levels >40% vol. corresponded, in 84% of the cases, to sampling dates when temperature was limiting. It is also expected since at very high moisture levels N<sub>2</sub>O is further reduced to N<sub>2</sub>.



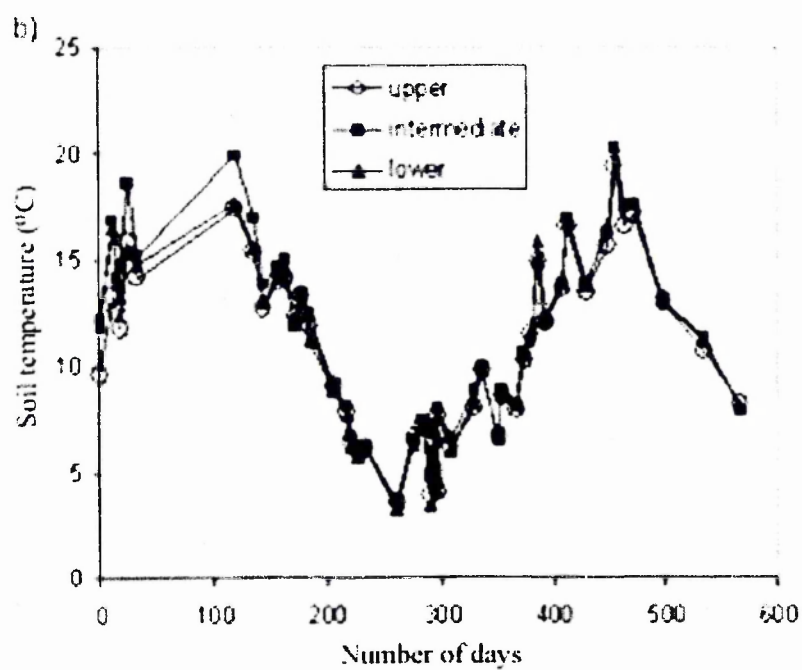
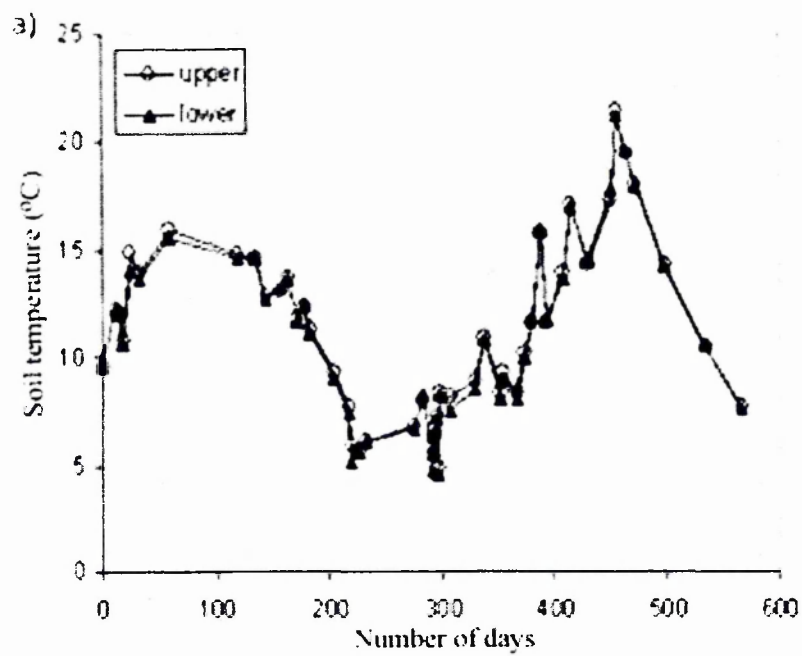


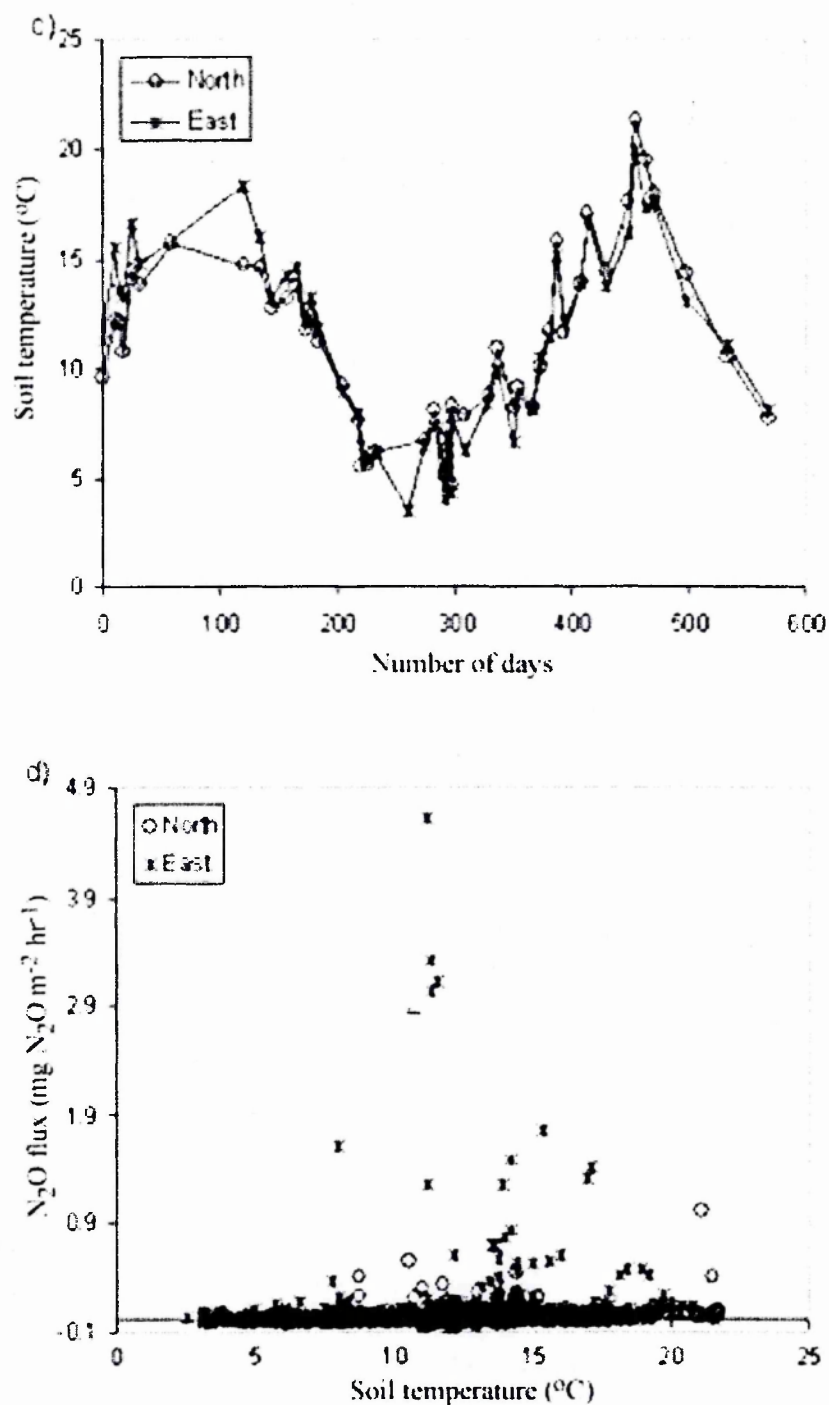
**Figure 3.4:** Mean water filled pore space (WFPS) at a) both experimental plots, b) the upper and lower location of the slope at Chicheley North, c) the upper, intermediate and lower locations of the slope at Chicheley East. d) Relationship between  $\text{N}_2\text{O}$  flux at both experimental plots and WFPS. Red dots in plots a) and b) represent measurements where there might have been a problem with the equipment.

### 3.4.3. SOIL TEMPERATURE

The soil temperature at the upper slope location at Chicheley North was generally slightly higher than that at the lower slope location (Fig 3.5a). This has been observed in the tropics where the wetter locations are cooler (Veldkamp *et al.*, 1998). However, in the U.K., water tends to dampen extreme events resulting in warmer soils in winter and cooler in summer. Soil temperature trends at Chicheley East were not as clear (Fig 3.5b). As with moisture, the soil temperature at the two experimental plots did not differ significantly, (Fig 3.5c). However, a non-parametric T-test (Mann-Whitney) done on the soil temperatures for both experimental sites confirmed that the soil temperatures were significantly ( $p < 0.0001$ ) higher when soil moistures were lower than 25 % vol. No linear relationships were found between  $N_2O$  fluxes and soil temperature (Fig 3.5d). However, like soil moisture, a clear non-linear pattern was observed in this relationship.  $N_2O$  fluxes were very low or negligible until a "threshold" was reached for soil temperature (8°C) above which fluxes were observed on some occasions (Fig 3.5d). A non-parametric T-test (Wilcoxon test) showed that fluxes were significantly higher for temperatures above 8°C than below ( $p < 0.001$ ). In 10 % of the cases where no  $N_2O$  emission was observed above the temperature threshold, the moisture threshold was not reached. In 29 % of the cases where no  $N_2O$  emission was observed above the temperature threshold the moisture exceeded 35 % vol., and  $N_2O$  was probably further reduced to  $N_2$ . Such thresholds (soil moisture and temperature) seem to be necessary for the potential emission of  $N_2O$  to occur. However, even when both thresholds are exceeded there are cases when no  $N_2O$  fluxes are observed.







**Figure 3.5:** Mean soil temperature at a) the upper and lower location of the slope at Chicheley North, b) the upper, intermediate and lower location of the slope at Chicheley East. c) Mean soil temperature at both experimental plots, on some occasions some differences were observed between sites due to sampling at different times of the day. d) Relationship between N<sub>2</sub>O flux at both experimental plots and the soil temperature measured at a depth of 10cm.

Maximum N<sub>2</sub>O emissions were measured at soil temperatures of 10°C to 12°C and soil moisture of 32% vol. to 36% vol. Above these levels, fluxes started to decline. However, we hypothesise that temperature and moisture will only have an effect on emissions when the other main soil parameters are not limiting. Dobbie *et al.* (1999) measured N<sub>2</sub>O emissions over 3 years at sites under intensively managed ryegrass (*Lolium perenne* L.) in the south east and southwest of Scotland in areas of contrasting climatic regimes, and at arable sites in an area of intermediate rainfall located on the drier east coast. They showed that the key factors controlling N<sub>2</sub>O emissions for N-fertilised soils are soil WFPS, soil temperature and soil particles mineral N concentration. Their results show that when frozen soils are thawing the basic temperature relationship ( $Q_{10} = 8.3$ ) is overridden by what is called the freeze/thaw effect. They suggested that this was probably due to increased denitrification in the uppermost soil layer induced by the increased availability of organic matter released on thawing of the frozen soil. They also found that there is a critical level of nitrate (5 mg NO<sub>3</sub><sup>-</sup> - N kg<sup>-1</sup> dry soil) in the soil below which N<sub>2</sub>O emissions may be very much reduced, even though the WFPS may be high. The decrease in flux rates observed at Chicheley when soil temperatures reached 18°C occurred in 80% of the cases at soil moistures below the threshold of 24% vol. and corresponds to the summer when conditions were drier and soil moisture was limiting. It is also expected that at the highest levels of temperature and moisture N<sub>2</sub>O is further reduced to N<sub>2</sub>.

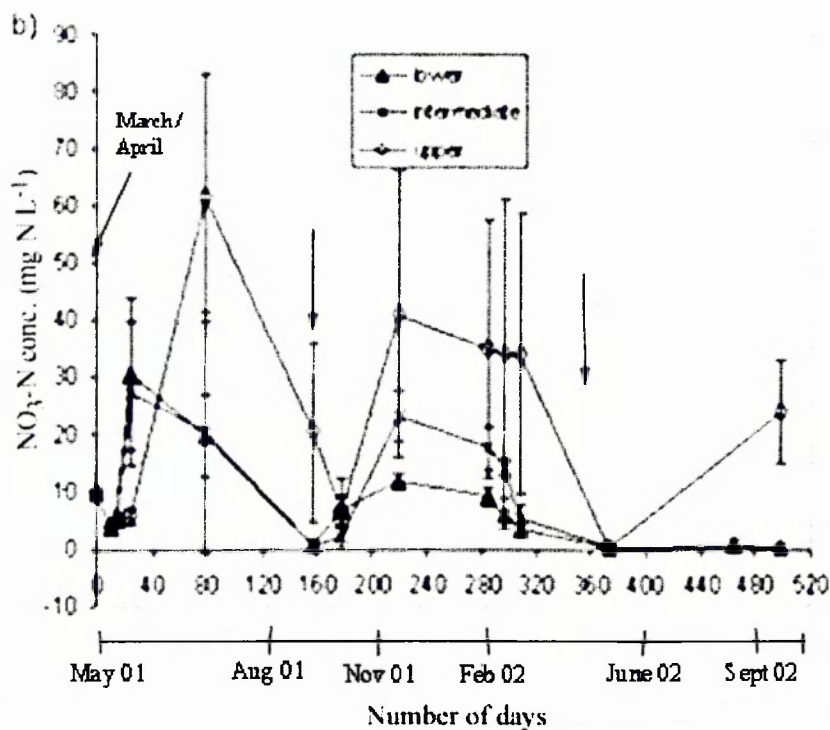
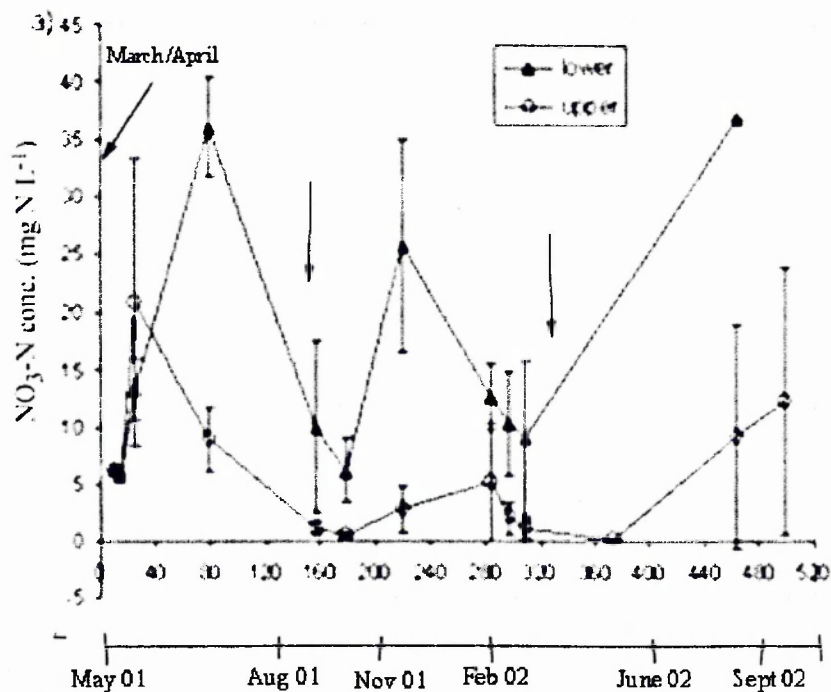
Previous studies have shown that N<sub>2</sub>O fluxes were related to soil temperatures. Smith *et al.* (1998) showed a steep increase in N<sub>2</sub>O emissions for soil temperatures between 5 and 11°C in a field study of the effect of N addition and diurnal changes in temperature. Other studies showed that substantial N<sub>2</sub>O emission occurred when temperatures increased for many soils in temperate climates (Skiba *et al.*, 1998; short-term measurements carried out at 22 sites). Most of these studies applied linear regressions, which are not appropriate for modelling N<sub>2</sub>O. In contrast, Butterbach-Bahl *et al.* (2002a), in a study of two forest soils (one spruce site and one beech site at the

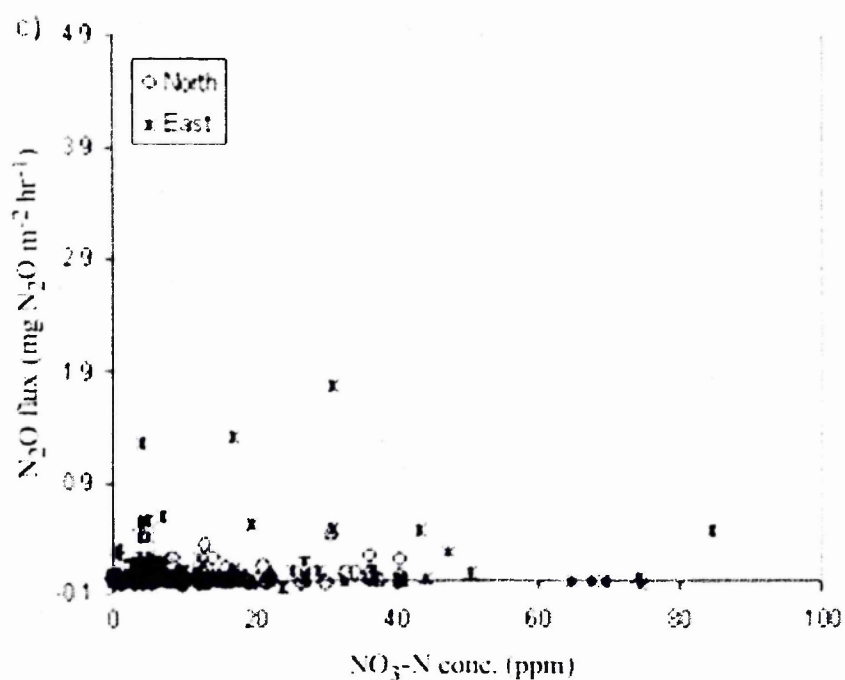
Högwald forest, Germany), could not demonstrate any relationship between  $\text{N}_2\text{O}$  emission and soil moisture or temperature due to the heterogeneity of denitrification activity in different soil cores taken from these forest sites and subjected to temperature and moisture manipulations. Their experiment was done on intact soil cores, including the organic layer and the top 0.15m of the mineral soil, directly transferred to the laboratory where temperature and soil moisture were manipulated. However, they found that at soil temperatures  $>6.5^\circ\text{C}$  a correlation between N input and  $\text{N}_2\text{O}$  fluxes was more pronounced than that at  $t < 6.5^\circ\text{C}$  (Butterbach-Bahl et al., 2002b). This suggests a temperature threshold existed for the soils they studied.

#### 3.4.4. SOIL NITRATE CONTENT

Figure 3.6a and b show the changes in  $\text{NO}_3^-$  concentration in the soil solution at both locations and along the gradient of the slope. The ammonium content of the soil solution over the study period was constantly close to the detection limit and so the  $\text{NO}_3^-$  data reflect the concentrations of dissolved inorganic N.  $\text{NO}_3^-$  in soil was generally plentiful so that it had no limiting effect on the  $\text{N}_2\text{O}$  fluxes. More  $\text{NO}_3^-$  was measured in the soil solution at Chicheley East, potentially reflecting the greater application rates of fertilisers. There was some evidence of a decrease in  $\text{NO}_3^-$  over the season, with some short-term increases following fertiliser application. At Chicheley East, the  $\text{NO}_3^-$  concentration in the soil solution decreases from the top to the bottom of the slope, suggesting that  $\text{NO}_3^-$  is removed from percolating water as it moves down slope before it reaches the stream. This is in agreement with findings from previous studies of riparian ecosystems (Ambus and Christensen, 1993; Smith and Duff, 1988). However, this pattern is reversed at Chicheley North. There could well be a source of  $\text{NO}_3^-$  between the upper and lower slope locations at Chicheley North. It is possible that the groundwater from the field is by-passing the upper slope location, resulting in higher

nitrate concentrations at the lower slope location. Figure 3.6c presents the relationship between  $\text{N}_2\text{O}$  fluxes at both experimental sites and the soil solution nitrate concentrations. No clear relationship was found, suggesting that there was always enough nitrate in the soil during the sampling period and that nitrate was not limiting.





**Figure 3.6:** Nitrate-N mean concentrations in soil solution (with standard errors) measured for (a) the upper and lower positions of the slope at Chicheley North and (b) the upper, intermediate and lower positions of the slope at Chicheley East. The black arrows indicate the different times of fertiliser application: March/April 2001, Sept/Oct 2001 and March/April 2002. Number of days is from 30/04/2001 (day0) to 11/09/2002 (day 499). c) Relationship between  $\text{N}_2\text{O}$  flux at both experimental plots and the soil solution nitrate concentration.

### 3.4.5. RAINFALL EVENTS

At both sites, we usually observed large pulses of  $\text{N}_2\text{O}$  following total daily rainfall events  $\geq 10$  mm (Fig 3.2). This effect only occurred when other factors controlling denitrification were above the thresholds identified previously. The proportion of  $\text{N}_2\text{O}$  emitted 2 days after major rain events accounted for 70 – 90 % and 80 – 90 % of the total annual  $\text{N}_2\text{O}$  at Chicheley North and Chicheley East, respectively. Others (e.g. Davidson and Swank, 1986; Ashby et al., 1998) have also observed a dramatic increase in  $\text{N}_2\text{O}$  emissions immediately after precipitation. The reason for this may be that soil moisture directly stimulates microbial activity. Nitrate can also accumulate in soil that is drying (Davidson *et al.*, 1990) as mineralisation occurs and be released as  $\text{N}_2\text{O}$  when dissolved carbon becomes available e.g., during rain events (Davidson *et*

*al.*, 1987). There may also be a piston effect, with rainwater pushing out N<sub>2</sub>O trapped in the soil.

### 3.5 Conclusion

N<sub>2</sub>O fluxes from the Chicheley riparian sites were highly variable both temporally and spatially. Fluxes were related to levels of soil moisture and soil temperature, but not simply; no linear relationships existed. However, a significant threshold response of N<sub>2</sub>O fluxes was observed at 8°C soil temperature and a similar threshold at 24% moisture content by volume is inferred. The thresholds appear necessary but not sufficient for N<sub>2</sub>O emission to occur. NO<sub>3</sub><sup>-</sup> content in the soil solution was always sufficient and was not a limiting factor at the sites. High pulses of N<sub>2</sub>O were usually generated following main rainfall events providing the other factors influencing the fluxes were not limiting. These results suggest that multiple controls exist on N<sub>2</sub>O emissions and non-linear relationships, perhaps with a hierarchical structure, are needed to model these emissions from riparian ecosystems. It also suggests that emission factors, although useful to estimate current rates and changes in N<sub>2</sub>O emissions, could introduce great uncertainties and lead to an important underestimation (perhaps of the order of 30 %) of such emissions if they are not taking into account N<sub>2</sub>O sources like riparian ecosystems.

## **Chapter Four**

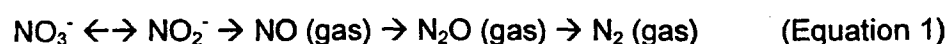
### **Denitrification and Denitrifying Enzyme Activity studies at the experimental sites**

(Part of this chapter is published in: Machefert S.E. and Dise N.B., 2005, Hydrological controls on denitrification in riparian ecosystems. *Hydrology and Earth System Sciences*, 2004, 8(4), 686-695)

#### **4.1 Denitrification rates and controls in riparian ecosystems**

##### **4.1.1 Introduction**

Denitrification is a biological process by which nitrogen is transferred from the soil to the atmosphere. This transfer is one of the principal mechanisms by which the environmental pollutant nitrous oxide (N<sub>2</sub>O) enters the atmosphere (Bouwman, 1990). NO and N<sub>2</sub>O are intermediate products of denitrification which may be further reduced to nitrogen gas. The net chemical reaction is:



Only trace amounts of nitric oxide (NO) are usually produced, and the main products are nitrous oxide (N<sub>2</sub>O) and dinitrogen (N<sub>2</sub>), with different suites of micro-organisms



involved in  $\text{NO}_2^-$  reduction and  $\text{N}_2\text{O}$  reduction.  $\text{N}_2\text{O}$  is an important greenhouse gas: it contributes approximately 6% of the total effect of all anthropogenically-enhanced greenhouse gases to global warming (Denmead, 1991) and has a radiative forcing 180 times greater than  $\text{CO}_2$  (Mogge *et al.*, 1998). However, denitrification in riparian soils may have the positive effect of reducing the hydrologic export of reactive N to the river channel, especially in agricultural catchments that receive high loads of fertiliser N.

The first investigations of denitrification (Wijler and Delwiche, 1954; Nommik, 1956) focused on the individual effects of pH,  $\text{NO}_3^-$  concentration, the presence of an available energy source, soil water and temperature. Since then, research has intensified to determine relationships between denitrification and ranges of these driving variables, especially moisture content or water-filled pore space (defined with other hydrologic terms in Appendix IV) (Robertson and Tiedje, 1984; Davidson and Swank, 1986; Groffman *et al.*, 1991). The experimental study presented in this chapter investigates the effects of soil water content on denitrification and the partition between the end products  $\text{N}_2$  and  $\text{N}_2\text{O}$  in the field. Very few long-term studies of denitrification in the field have been undertaken, and of these, none to our knowledge have been carried out *in situ* – that is, studying the denitrification rates and environmental controls from intact ecosystems rather than cores placed in the field (Tiedje *et al.*, 1989).

This study was undertaken to identify relationships between denitrification and environmental factors to ultimately develop a catchment-scale model able to simulate nutrient processes in the riparian zone. The INCA model (Integrated Nitrogen Model for European CAatchments; Whitehead *et al.*, 1998; Chapter 5) currently simulates denitrification but it was necessary to determine whether it is adequately represented, and if not, how the simulation of denitrification in the riparian zone could be improved. The present representation of denitrification within INCA is a function of a soil temperature-dependent parameter and two controlling factors: soil moisture deficit (SMD) and soil nitrate concentration (Equation [17], in Wade *et al.*, 2002). The value of

the parameter is determined by calibration. A soil moisture threshold is also set by calibration in INCA.

The following questions are addressed in this chapter:

- (a) What are the most important factors controlling the denitrification rate in riparian zones receiving nitrate-rich agricultural runoff?
- (b) Can a simple mathematical relationship be developed to describe these processes?
- (c) Is the relationship derived using data collected *in situ* where the soil remained intact supported by the results of other studies, where the measurements have been made in the lab and in the field on cores?
- (d) Can these relationships be generalised into an equation or set of equations for use with INCA, or other models of riparian nutrient dynamics?

#### 4.1.2 Materials and methods

##### STUDY AREA

The experimental ( $C_2H_2$ ) plot is within the Great Ouse river catchment (Chapter 2, Fig 2.) in the United Kingdom. It is located near the town of Chicheley, northeast of the city of Milton Keynes (52.33° N, 0.70° W). The site is a riparian ecosystem situated in an active farm and drains an agricultural field into a small stream: the Chicheley Brook. The plot is characterised by a gradual slope (17%) to the stream and a long runoff from the field. The soil is from the Fladbury Series, a grey clayey pelo-alluvial gley with >50% clay in the plough layer (0-25 cm), and the soil pH (in  $H_2O$ , 1:2.5) is about 7.6.

Ten 'static' chambers were installed at the site. The chambers were made of 30 cm diameter PVC rings inserted 5 cm deep in the topsoil at three levels above the stream surface. Four replicate chambers were installed near the stream, and three each at an intermediate zone and upslope (Chapter 2, Fig. 6). N<sub>2</sub>O fluxes were determined using the closed chamber technique (Hutchinson and Mosier, 1981). Three gas samples (20 mL each) were withdrawn from the headspace using a 60 mL syringe (a) immediately after closing the chamber, (b) 30 minutes and (c) 60 minutes later. Before removing a sample in each case, the atmosphere in the chamber was mixed by pumping the syringe plunger 6 times. Each sample was injected into an evacuated container (Labco Exetainer, 10 mL).

The change in N<sub>2</sub>O concentration as a function of time was used to calculate the emission rate of N<sub>2</sub>O. After the first 60-minute sampling period, the chambers were left open for 30 minutes to allow the atmosphere of the chamber to return to ambient levels of N<sub>2</sub>O. After that, the chambers were closed again and acetylene was added in the headspace to reach 10% of the volume delimited by the chamber. Such a level of acetylene inhibits the reduction of N<sub>2</sub>O to N<sub>2</sub> in the final step of the denitrification pathway (Equation 1). The acetylene was left to stand for two and a half hours, and three and a half hours following rainfall, to allow diffusion into the soil (Hutchinson and Mosier, 1981). After that time, the chambers were aerated for another 30 minutes before a second measurement of N<sub>2</sub>O fluxes was taken following the procedure described in the previous paragraph. The amount of N<sub>2</sub>O produced in the chamber's atmosphere after the addition of acetylene corresponded to the total rate of denitrification (N<sub>2</sub> + N<sub>2</sub>O). Ambient air samples were also taken at each visit.

The Exetainers were transported to the laboratory and N<sub>2</sub>O concentrations were determined using a gas chromatograph fitted with an electron capture detector and equipped with a PorapakQ, 50-80, 6ft column. The carrier gas (N<sub>2</sub>) flow rate was 58

mL min<sup>-1</sup>, the detector temperature was 320 °C, and the injector and the oven were at 45 °C and 60 °C, respectively.

The soil moisture content (% vol. TDR, 6 cm probe) was monitored on each sampling date. The soil temperature (10 cm deep) was also monitored. Total denitrification was measured on 8 occasions during the period from 08/03/2002 to 09/04/2003 in each of the ten chambers.

## DATA ANALYSES

Multiple regression analysis is used to investigate potential linear relationships between several independent or predictor variables and a dependent or criterion variable. In general, multiple regression allows the researcher to ask the general question "what is the best predictor of ...". However, it entails a number of assumptions. First of all, as is evident in the name multiple *linear* regression, it is assumed that the relationship between variables is linear. In practice this assumption can rarely be confirmed; fortunately, multiple regression procedures are not greatly affected by minor deviations from this assumption. Where the linearity is not confirmed, the data can be transformed using the logarithm (e.g. log or ln) in order to make the distribution linear.

It is also assumed in multiple regression that the residuals (predicted minus observed values) are distributed normally (i.e., follow the normal distribution). Again, even though most tests (specifically the *F*-test) are quite robust with regard to violations of this assumption, it is *always* a good idea, before drawing final conclusions, to review the distributions of the major variables of interest.

In this study, the data were analysed by stepwise multiple regression and fitting empirical equations between denitrification rates and the relevant environmental factors. These results were compared to those from other studies from the literature. Previously, Machefert *et al.* (2004) showed that soil moisture was the main driving control on N<sub>2</sub>O

emission from Chicheley, with soil temperature and dissolved organic carbon of secondary importance. Nitrate was rarely if ever limiting in this well-fertilised site. We therefore place special emphasis on the relationship between soil hydrology and denitrification in the current study.

## COMPARISON WITH OTHER STUDIES

Six studies were used for comparison with our findings, with focus on experiments undertaken in riparian ecosystems, whether they were forested, herbaceous or agricultural. The particular experiments were chosen because they were studies of denitrification and its controls (field or lab experiment on soil cores) and because they were long-term experiments (at least 12 months) or were a compilation of the results from a large number of samples analysed. As such, these studies provide a comparison with the measurements made *in situ* at Chicheley where the soil remained intact. The denitrification rates in all six studies were determined using the acetylene inhibition technique (Tiedje, 1994; Yoshinari *et al.*, 1977). The soil moisture was expressed as water-filled pore space and gravimetric moisture content in five of the studies.

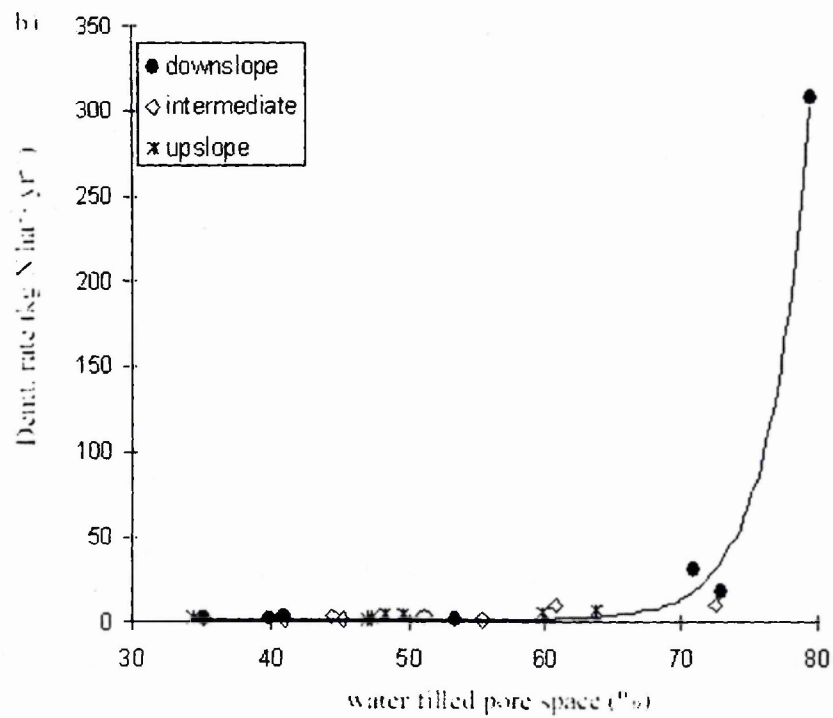
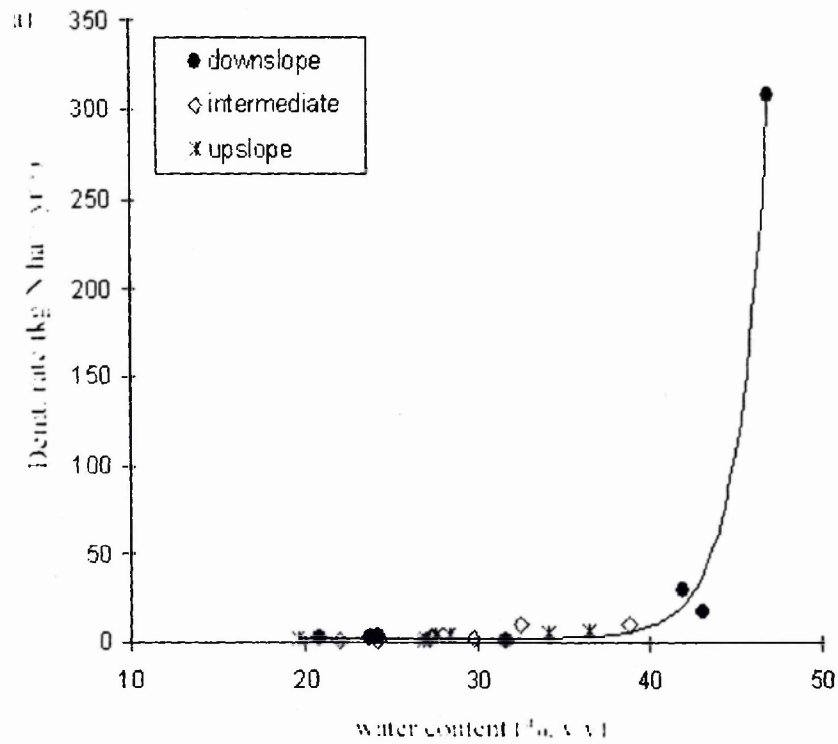
The soil moisture in the sixth study considered was expressed as percentage of the water holding capacity (% WHC) and could not be changed to volumetric soil moisture content or water filled pore space. The data obtained in this study were based on sieved soil samples that were incubated in flasks. These could not be related to the WFPS of intact soil cores.

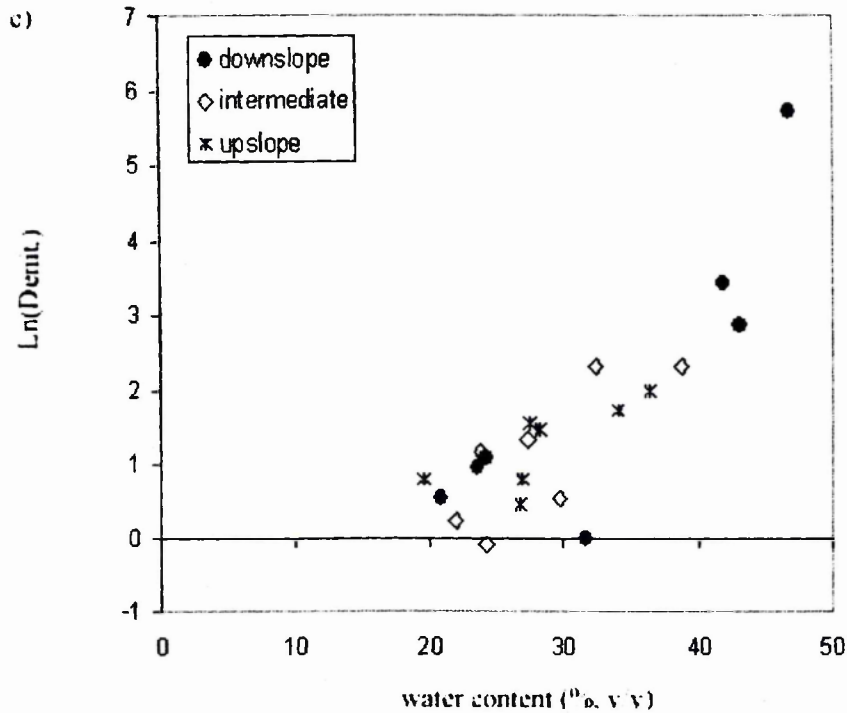
#### 4.1.3 Results and discussion

##### RIPARIAN STUDY

Total denitrification rates at Chicheley were highest at the lower level nearest to the stream, and decreased uphill away from the stream. This is in agreement with previous findings (Davidson and Swank, 1986; Groffman and Tiedje, 1989) and expected, since rates of denitrification are known to be higher at higher moisture contents (Davidson, 1991). The soil at the lower level was always wet and sometimes waterlogged. Regular flooding events contributed to maintaining the high moisture content of the lower soil. During the denitrification measurements, the soil temperature range was 4.9 - 19°C. The soil temperature was 7.3°C and 5.1°C when denitrification was highest and lowest, respectively.

Figure 4.1 shows the relationship between denitrification rates and volumetric water content (4.1a) and between denitrification and water filled pore space (4.1b). Figure 4.1c shows a plot of the natural logarithm of denitrification rate in relation to the water content of the soil studied ( $r^2 = 0.84$ ). In Figs 4.1a and 4.1b, an exponential model was fitted to the experimental data, with a coefficient of determination of 0.99 for both models ( $y = 1.93E-09e^{0.549x} + 2.333$  and  $y = 7.6E-10e^{0.337x} + 1.08$ , respectively). This confirms that a threshold of moisture of about 65 – 70% (WFPS) has to be reached for high denitrification rates to occur and illustrates that the relationships are not linear but WFPS is nonetheless important.





**Figure 4.1:** Plot of total denitrification ( $\text{kg N ha}^{-1} \text{ yr}^{-1}$ ) against (a) volumetric soil moisture (%) and (b) water filled pore space (%WFPS) at the three different levels of the slope at the Chicheley study site. Exponential (curve) is fitted to the whole of the data with an  $r^2 = 0.99$ . c) Natural logarithm of total denitrification against volumetric soil moisture (%) at all three different levels of the slope at the Chicheley study site.

Water-filled pore space, soil nitrate, dissolved organic carbon (DOC) and soil temperature were included in a step-wise regression on the  $\text{N}_2\text{O}$  emissions and the natural logarithm of denitrification rates. This was done on the individual rates, the mean rates by chamber and the mean rates by level of the slope. The results are presented in Table 4.1. The equations representing (a) individual, (b) mean rates by chamber and (c) mean rates by level of the slope for  $\text{N}_2\text{O}$  fluxes and  $\ln$  (denitrification rates) are as follows:

$$(a) \text{N}_2\text{O}_{\text{flux}} = -3.83 \cdot 10^{-2} + 2.2 \cdot 10^{-3} \times t_{\text{soil}} + 4.8 \cdot 10^{-4} \times \text{WFPS}$$

$$(r^2 = 0.253, N = 27, p = 0.03)$$

$$\ln(\text{Denit}_{\text{rate}}) = -5.75 + 4.87 \cdot 10^{-2} \times \text{WFPS}$$

$$(r^2 = 0.318, N = 27, p = 0.002)$$



$$(b) \text{ N}_2\text{O}_{\text{flux}} = - 7.2 \cdot 10^{-2} - 2.54 \cdot 10^{-3} \times t_{\text{soil}} + 2.98 \cdot 10^{-3} \times \text{WFPS}$$

$$(r^2 = 0.610, N = 10, p = 0.037)$$

$$\text{Ln} (\text{Denit}_{\text{rate}}) = - 12.4 + 0.2 \times \text{WFPS}$$

$$(r^2 = 0.208, N = 10, p = 0.186)$$

$$(c) \text{ N}_2\text{O}_{\text{flux}} = - 7.96 \cdot 10^{-2} + 9.5 \cdot 10^{-4} \times \text{WFPS} + 3.51 \cdot 10^{-3} \times \text{NO}_3^- \text{ soil}$$

$$(r^2 = 0.821, N = 9, p = 0.006)$$

$$\text{Ln} (\text{Denit}_{\text{rate}}) = - 6.5 + 6.4 \cdot 10^{-2} \times \text{WFPS}$$

$$(r^2 = 0.618, N = 9, p = 0.012)$$

Following each equation, in brackets, are the total coefficient of determination  $r^2$  as well as the number of data points  $N$  and the probability level  $p$ . The distribution of the residuals for each case is presented in Appendix X. The results show that water-filled pore space (%) is the main predictor of both  $\text{N}_2\text{O}$  emissions and denitrification rates at our sites, except in the case of the mean-by-chamber dataset where the relationship between  $\text{Ln} (\text{Denit}_{\text{rate}})$  and WFPS is not significant.

		<b>N<sub>2</sub>O emissions</b>	<b>Natural logarithm of Denitrification rates</b>
<b>(a) Individual dataset</b>	predictor 1 $r^2$ p	water-filled pore space 0.253 (cumulative) 0.029	water-filled pore space 0.318 0.002
	predictor 2 $r^2$ p	soil temp. °C 0.086 0.009	
<b>(b) Mean-by-chamber dataset</b>	predictor 1 $r^2$ p	water-filled pore space 0.610 (cumulative) 0.034	water-filled pore space 0.208 0.186
	predictor 2 $r^2$ p	soil temp. °C 0.222 0.018	
<b>(c) Mean-by-level dataset</b>	predictor 1 $r^2$ p	water-filled pore space 0.821 (cumulative) 0.003	water-filled pore space 0.618 0.012
	predictor 2 $r^2$ p	soil nitrate 0.153 0.002	

**Table 4.1:** Stepwise regression results on nitrous oxide emission rates and the natural logarithm (ln) of denitrification rates at the acetylene site. The dependent variables considered for the regressions were water-filled pore space, soil temperature, soil nitrate content and dissolved organic carbon. The results are presented for 3 different datasets: Individual dataset, mean-by-chamber dataset and mean-by-level dataset.

The moisture threshold between N emitted as N<sub>2</sub>O and N emitted as N<sub>2</sub> was determined at all three levels. For soil volumetric water contents below 35%, gaseous N was emitted as 50% N<sub>2</sub>O and 50% N<sub>2</sub> at the lower (N = 4), intermediate (N = 5) and

upper ( $N = 6$ ) levels above the stream surface. Above this moisture level, the partition was 80%  $N_2$  and 20%  $N_2O$ , 90%  $N_2$  and 10%  $N_2O$  and 60%  $N_2$  and 40%  $N_2O$ , at the lower ( $N = 4$ ), intermediate ( $N = 3$ ) and upper ( $N = 2$ ) level respectively. However, there was a large range on these estimates.

Annual denitrification fluxes of  $5.0$  and  $4.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  were estimated at the intermediate and upper levels respectively, farthest from the stream surface where the moisture status of the soil was not significantly different. Similar results have been reported for other riparian ecosystems of low moisture status or low nitrate (e.g. Robertson and Tiedje, 1984). Annual denitrification at the near-stream site was  $71.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , about 15 times higher than the two sites higher up the slope. This is due to a single high denitrification rate measured at the near-stream site. If this value is ignored, the annual denitrification at the near stream site would still be 4 to 5 times higher than that at the intermediate and upper sites. However, from comparison of these results to other studies (next section), this point should not be ignored - it corresponds to the denitrification rates expected at higher WFPS ( $> \text{ca } 70\%$ ).

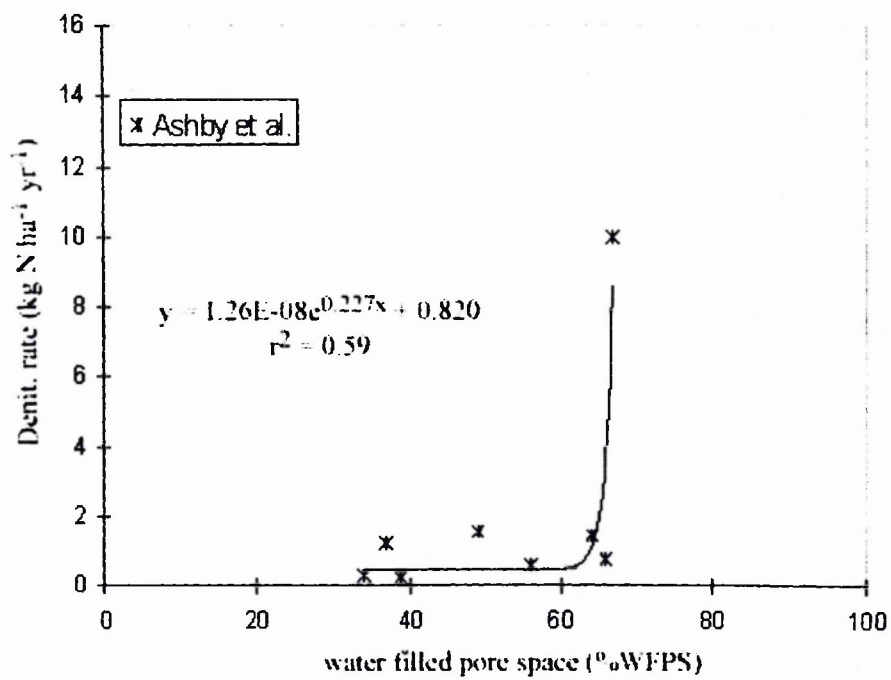
## COMPARISON WITH OTHER STUDIES

Ashby *et al.* (1998) reported a laboratory study of denitrification and its controls in riparian soils of three catchments located in the Catskill mountains, U.S.A. (Fig. 4.2a). The catchments were forested and the primary cover type was sugar maple, beech and yellow birch (*Acer saccharum*, *Fagus grandifolia*, *Betula alleghaniensis*). Denitrification measurements were made as part of two soil surveys: (1) a surface survey including three poorly-drained surface soils and (2) a riparian sequence survey including both surface and sub-surface soils from stream-edge, stream-bank and upland locations. Denitrification rate was measured in the lab on intact soil cores (584) sampled on 12 dates from May through October 1994 and in April and June 1995. Soil samples that

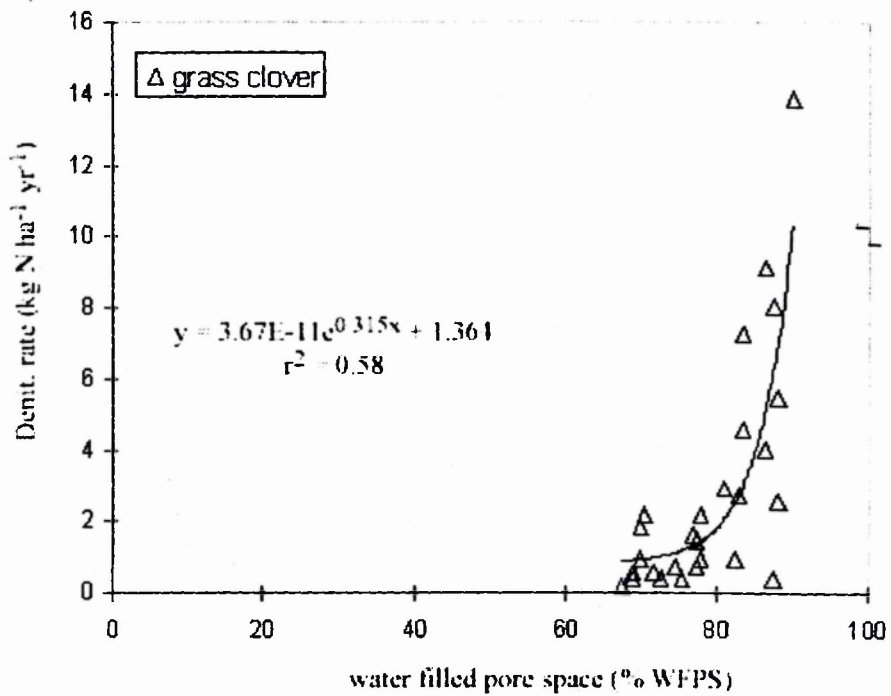
were collected in the spring, late summer and fall of 1994 were incubated at 8° to 11°C, whereas those collected in the summer of 1994 and 1995 were incubated at room temperature (21° to 24°C).

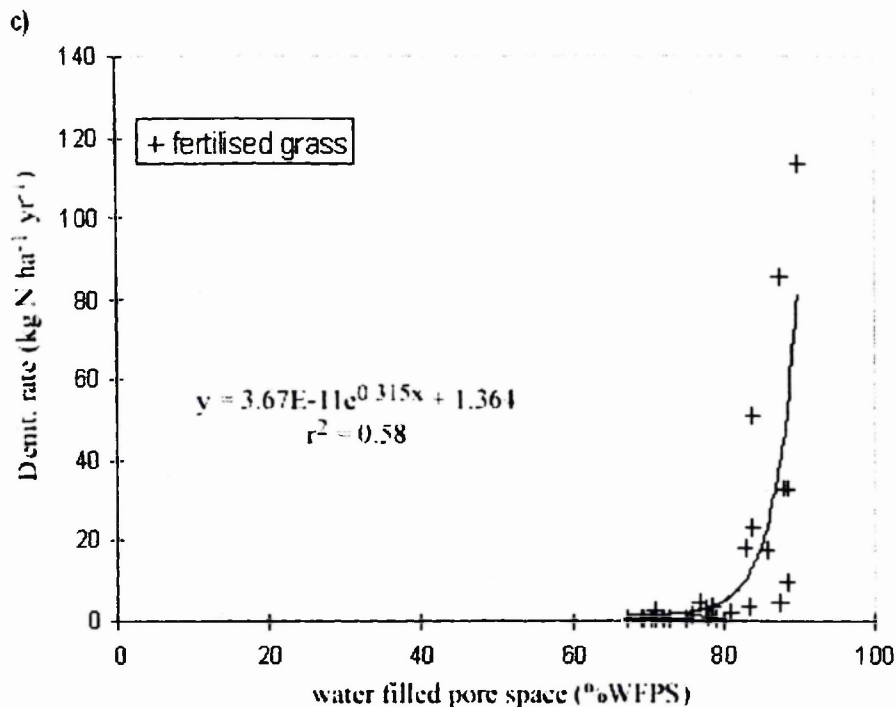
Figure 4.2a presents the median denitrification rate for 5 surface soils (seep, toeslope, stream-edge, stream-bank, and upland) and 3 deep soils (stream-edge, stream-bank, and upland; seep and toeslope were not analysed) in relation to the mean water-filled pore space. Volumetric soil moisture and denitrification rates were highest in seep and toeslope (saturated soils at the base of the slope) soils, and lowest in upland soils. The denitrification rate was significantly related to the soil moisture as % WFPS ( $p = 0.008$ ). Ashby *et al.* reported higher denitrification rates following precipitation but the limited data did not allow them to create any models.

a)



b)





**Figure 4.2:** Plot of total denitrification ( $\text{kg N ha}^{-1} \text{ yr}^{-1}$ ) against water filled pore space (%) (a) at the Ashby *et al.* (1998) site, (b) at the grass clover pasture in Ruz-Jerez *et al.* (1994) and (c) at the fertilised grass pasture described in Ruz-Jerez *et al.* (1994). Curves are the exponential equations fitted to each dataset, equations and coefficients of determination are given on each plot.

Ruz-Jerez *et al.* (1994) reported a long-term measurement of denitrification in contrasting pastures: unfertilised perennial ryegrass/white clover (Fig. 4.2b) and fertilised ryegrass sward receiving  $400 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (Fig. 4.2c). In this experiment, total denitrification was determined using the soil core incubation system under field conditions (Ryden *et al.*, 1987). The measurements took place between 12 September 1989 and 31 May 1991. The soil temperature during the study ranged from  $10^\circ$  to  $24^\circ\text{C}$ .

Ruz-Jerez *et al.* (1994) observed a marked seasonal variation of denitrification, with the highest rates in late autumn and winter. In general, low rates of denitrification were reported during spring and summer when temperatures were highest but soil moisture was below field capacity. When the soil moisture content was above field capacity for an extended period, denitrification rates increased. Another indication that the soil moisture played a significant role in controlling the rate of denitrification was that the

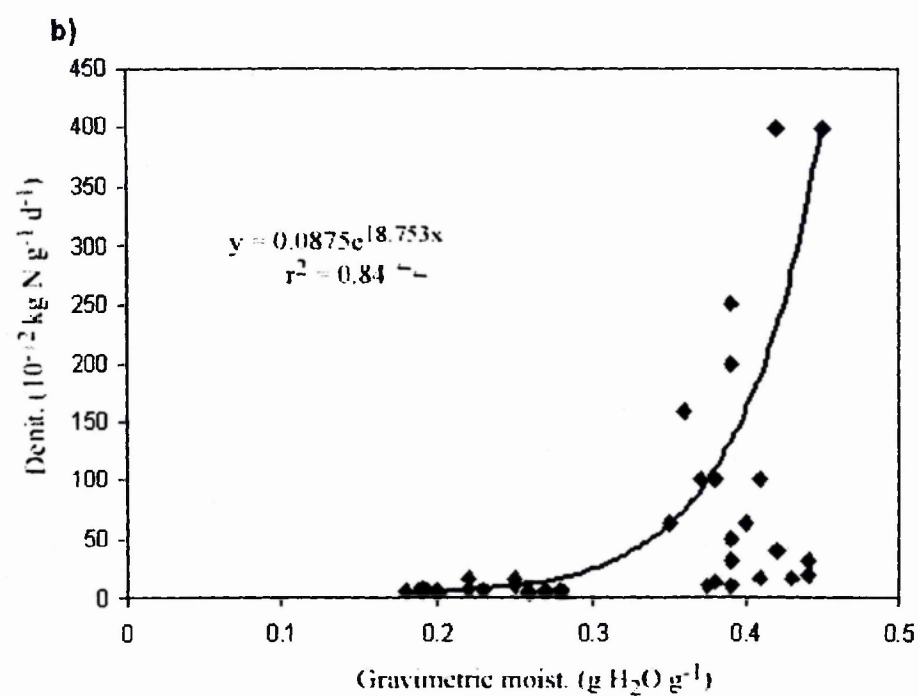
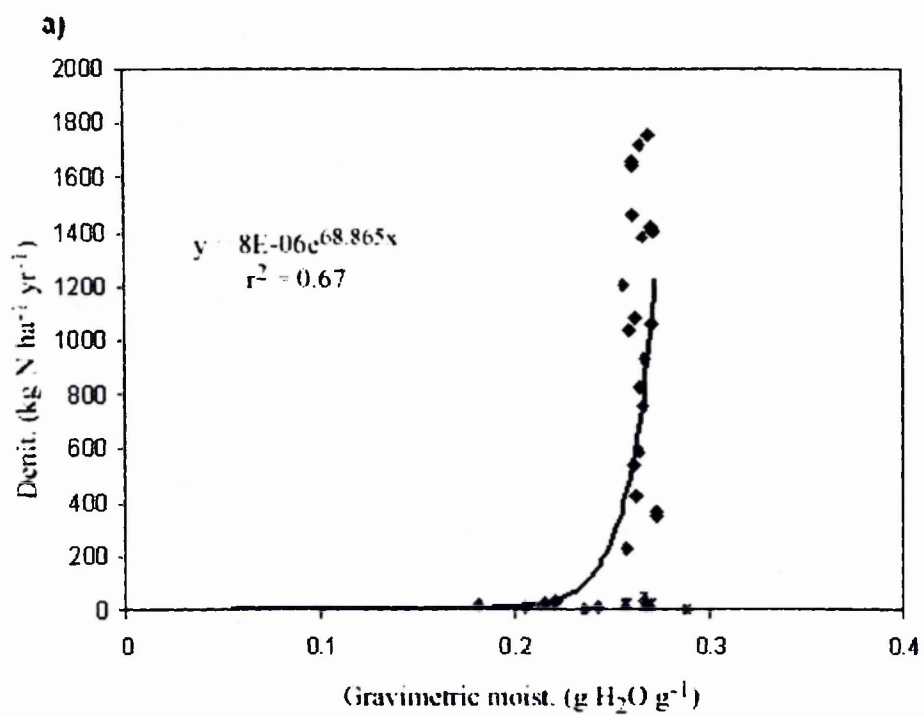
emission rate dropped when the soil moisture content fell below the field capacity in late winter. The rate of denitrification was much higher in the fertilised ryegrass pasture than the unfertilised site, probably due to the greater amount of nitrate available at the fertilised pasture (Ruz-Jerez *et al.*, 1994).

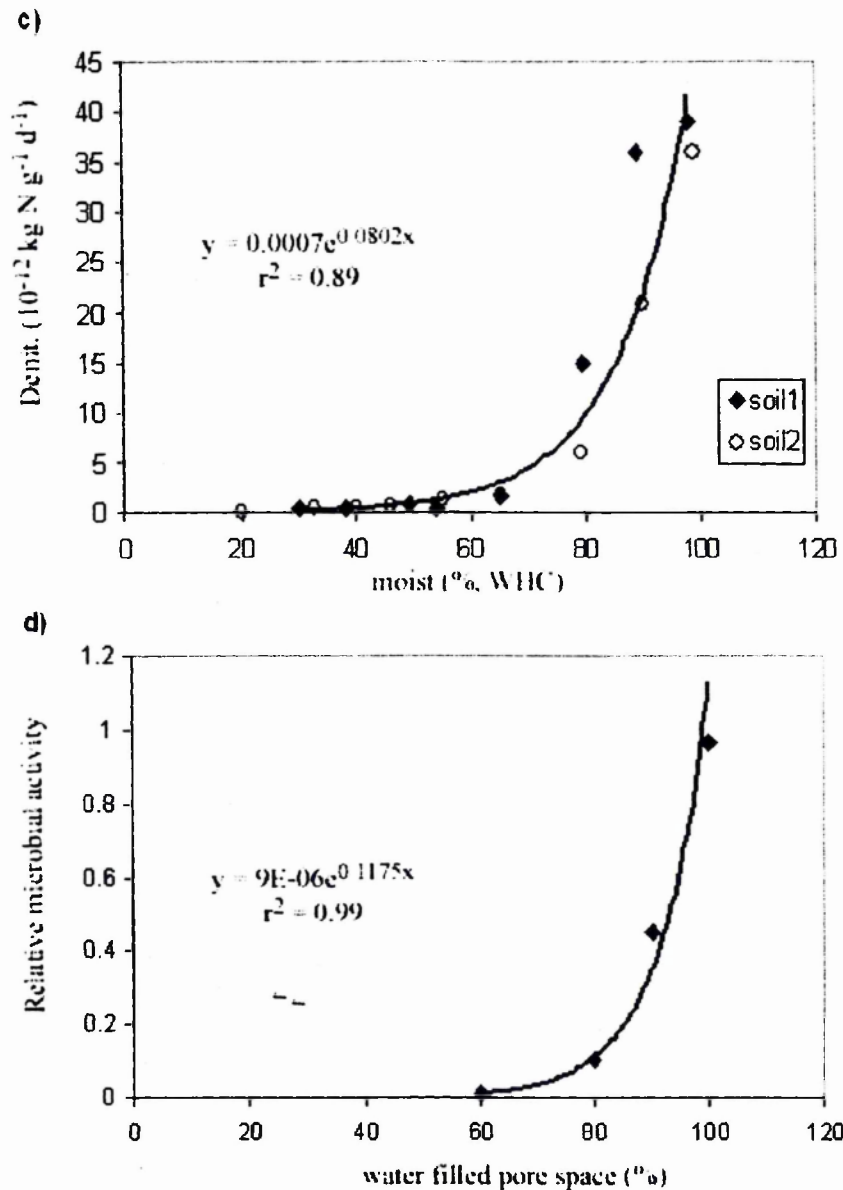
The results from the studies by Ashby *et al.* (1998) in forests, Ruz-Jerez *et al.* (1994) in fertilised and unfertilised pasture, and Chicheley (Fig 4.1b) in semi-natural streambank vegetation, measured at different temperatures, all show an exponential correlation between denitrification rate and water filled pore space, with a threshold WFPS at 65-85%. Results from the experiment presented in this thesis and done at an undisturbed 'intact' field site are similar to those of the other studies on soil cores incubated either in the lab or the field. The main difference between the studies is in the absolute rate of denitrification: it is about 20 times higher for Chicheley and the fertilised grass (Fig 4.2c) than for the two unfertilised sites. The difference in denitrification rates between the two sites from Ruz-Jerez *et al.* (1994) is a result of the higher concentrations of nitrate in the fertilised system. This is also the case at the Chicheley site which drains an agricultural field receiving large amounts of fertiliser, so that  $\text{NO}_3^-$  concentrations are not limiting. Ashby *et al.* (1998) commented that the relatively low denitrification rates in their study in relation to rates measured in other hardwood forests was probably due to the soil characteristics (well-drained upland soils with shallow O horizons). Thus WFPS, nitrate, and soil carbon are all identified as limiting in their study.

Four other studies of denitrification used parameters that could not be easily converted into either denitrification rate or water-filled pore space, but are nonetheless useful for this data compilation. Henault and Germon (2000) presented a study of NEMIS, a predictive model of denitrification on the field scale, and the three-year database associated with the model development (Fig. 4.3a). The study site was a field in Citeaux, France that had been cultivated for more than 50 years. During the

experiment, the field was cropped with winter wheat in 1991 (fertilised with 170 kg N ha<sup>-1</sup>), spring barley in 1992 (fertilised with 100 kg N ha<sup>-1</sup>) and winter wheat again in 1993 (fertilised with 170 kg N ha<sup>-1</sup>). Fertiliser was applied as ammonium nitrate. Denitrification was measured in the laboratory on untreated soil cores and cores subjected to various treatments. These included addition of 500 ml of deionised water over 24 h followed by drainage over 24 h, addition of 500 ml of a 0.1 M KNO<sub>3</sub> solution (drop by drop over 24 h followed by drainage for 24 h) and addition of a series of nitrate solutions (500 ml) of increasing concentrations (0 to 0.1 M) without drainage. The incubations were carried out at 20°C. The denitrification rates from the untreated cores ranged between 2.19 and 32.8 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The denitrification rates measured on the wetted cores (either water, or different concentrations of KNO<sub>3</sub>) were higher than those on untreated cores on each occasion, ranging from 7.3 to 1825 kg N ha<sup>-1</sup> yr<sup>-1</sup>.







**Figure 4.3:** a) Denitrification rates ( $kg\ N\ ha^{-1}\ yr^{-1}$ ) versus gravimetric soil moisture ( $g\ H_2O\ g^{-1}$ ) from Henault and Geron (2000). An exponential curve is fitted to the data (◆), excluding points corresponding to gravimetric moistures above 0.25 with denitrification rates below  $73\ kg\ N\ ha^{-1}\ yr^{-1}$  (○), wetted cores with lowest nitrate contents, b) Denitrification rates ( $10^{-12}\ kg\ N\ g^{-1}\ d^{-1}$ ) versus gravimetric soil moisture ( $g\ H_2O\ g^{-1}$ ) from Ettema *et al.* (1999). The curve is fitted to the sites where oxidisable C is not limiting. c) Denitrification rates ( $10^{-12}\ kg\ N\ g^{-1}\ d^{-1}$ ) versus soil water holding capacity (%) from Bollman and Conrad (1998) and d) Relationship between water filled pore space and relative microbial activity (interpreted as rate of denitrification by Mosier *et al.*, 2002) from Linn and Doran (1984). a) to d) The curves are exponential equations we fitted to each dataset. Equations and coefficients of determination are given on each plot.

Figure 4.3a shows the relationship between denitrification rates and gravimetric moisture content from this study. The data show a moisture threshold at 25% (w/w) below which denitrification rates are very low and above which most measurements

show high denitrification rates. An exponential curve was fitted to these data. There are a few cases of low denitrification rates (below  $73 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) at moisture values above 25%. These were all from the zero  $\text{NO}_3^-$  addition wetted cores. It is possible that in these cores nitrate was too limiting for denitrification to occur.

Ettema *et al.* (1999) studied the response to surface nitrogen input of a poorly-drained riparian soil and the temporal changes in denitrification rates. The experiment was carried out in a 50-year-old riparian forest bordering a small stream, located at the University of Georgia Coastal Plain Experimental Station in the Little River Watershed, USA (Fig. 4.3b). Denitrification rates were measured on soil increments (2.5 cm diameter) from microcosms receiving different levels of N-addition and incubated at the ambient water content. Incubations were carried out in the laboratory at  $25^\circ\text{C}$ . Soil moisture was determined gravimetrically. An exponential curve was fitted to those sites where the authors suggested oxidisable C was not limiting (Fig. 4.3b). Mean rates of denitrification in the control microcosms were 10-fold higher in the soils located closest to the stream, where moisture levels were significantly higher. N-addition hardly affected the low rates of the zone farthest from the stream, but significantly increased the rates measured at the near-stream zone (wetter sites).

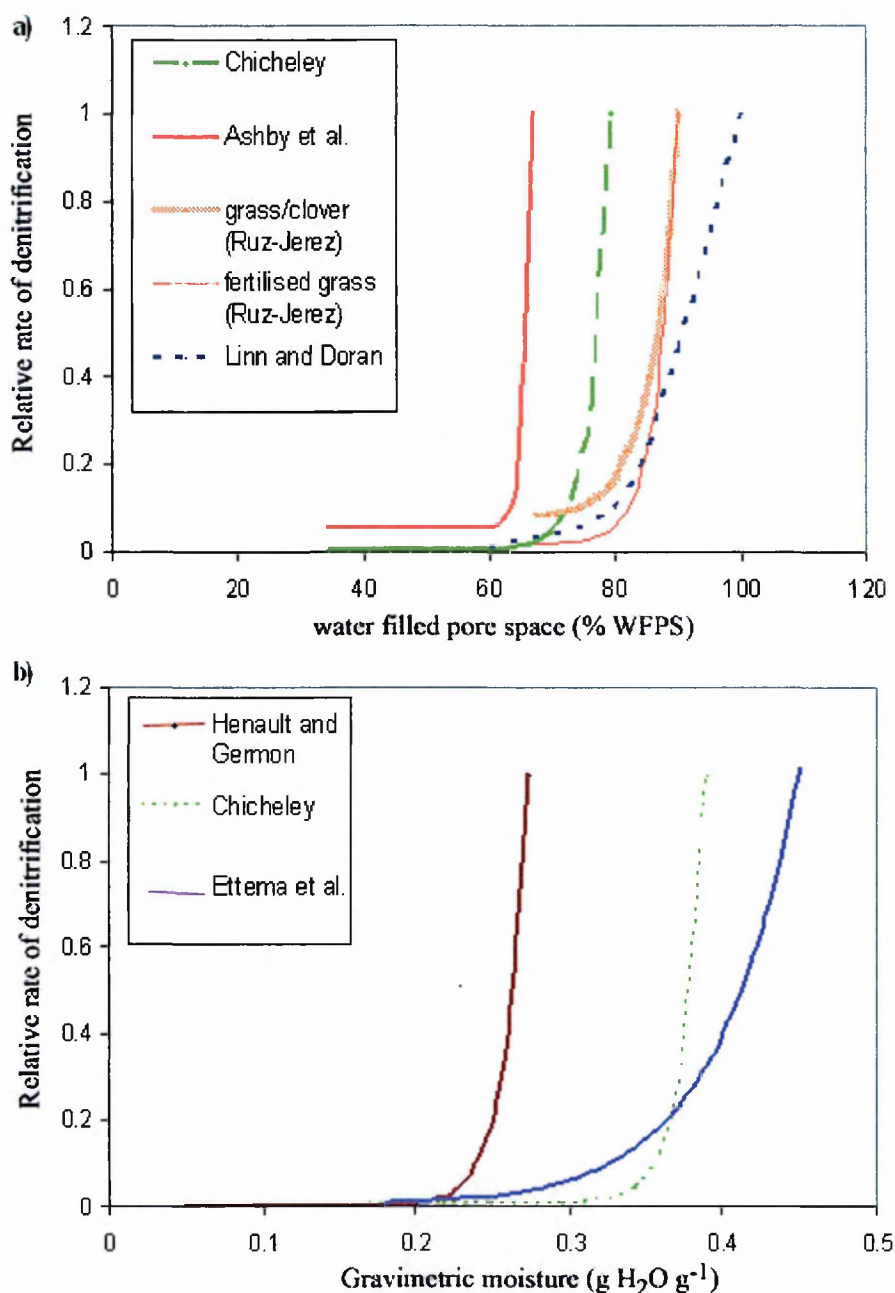
Bollman and Conrad (1998) studied the influence of  $\text{O}_2$  availability on  $\text{N}_2\text{O}$  release by denitrification in soils (Fig. 4.3c). They used sieved soil samples and it was not possible to calculate the soil volumetric moisture content or water filled pore space. Instead, water holding capacity (WHC, defined in Appendix IV) is used. The soil samples were incubated at  $25^\circ\text{C}$ . The two soils were agricultural soils, a luvisol (loamy silt) cropped with wheat (soil 1) and a cambisol (sandy silt) cropped with barley (soil 2). The analysis again showed that denitrification rates were exponentially related to soil moisture contents, with a threshold at approximately 70% WHC. Since both soils behaved similarly only one exponential curve was fitted to the two.

Finally, Mosier *et al.* (2002) described the role of denitrification in the nitrogen economy of crop production and the environment in an overview of denitrification in soils and how to manage it. They presented a general relationship between soil water-filled pore space and microbial denitrification rates, adapted from Linn and Doran (1984) who conducted a lab-incubation study (soil cores) to examine the effect of a range of WFPS values on soil microbial activity (Fig. 4.3d). The cores were incubated at 20° to 22°C. Mosier *et al.* (2002) concluded that the relative activity of anaerobic denitrification is negligible at 60% water-filled pore space but increases with increasing water and reaches a maximum at saturation. Their general relationship also shows a threshold for denitrification at approximately 70% WFPS.

Even though the soil moisture characterisation differed between these experiments and the study presented in this thesis, as well as the previous studies described (Ashby *et al.*, 1998 and Ruz-Jerez *et al.*, 1994), all results agree that denitrification rates are exponentially related to soil moisture. In addition, comparable studies (Fig. 4.2, Fig. 4.3) show approximately the same threshold values (60-80% WFPS; 25-30% gravimetric moisture) where denitrification rates increase exponentially.

To compare the different exponential curves fitted to the different datasets considered in this study, the rates of denitrification in each study were scaled to the maximum value measured (Fig. 4.4). In Figures 4.4a and 4.4b all curves have a similar shape, showing negligible rates of denitrification until a threshold value of the water-filled pore space or gravimetric moisture is reached, above which denitrification increases sharply. This value ranges between 60 and 80% of water-filled pore space and (less clearly) 20-40% of gravimetric moisture. The actual value within this range depends upon the complex interaction of factors such as antecedent rainfall, water residence time, or soil texture. All the studies described in figures 4.1-4 suggest, however, that moisture is the

main driving force determining the potential for denitrification, whereas the absolute rate of denitrification is determined by the available  $\text{NO}_3^-$  at the site and secondarily by temperature and DOC.



**Figure 4.4:** a) Exponential relationships between the relative rate of denitrification (no unit) and the water-filled pore space (% WFPS) for the following studies: Chicheley, Ashby *et al.* (1998), a grass/clover pasture (Ruz-Jerez *et al.*, 1994), a fertilised grass pasture (Ruz-Jerez *et al.*, 1994) and Linn and Doran (1984). The curves are redrawn from the exponentials fitted to the datasets from Figures 2b, 3 a-d and 4c and the different absolute amounts of denitrification are scaled to the maximum value measured. b) Exponential relationships between the relative rate of denitrification (no unit) and the gravimetric moisture content ( $\text{g H}_2\text{O g}^{-1}$ ) for the following studies: Henault and Geron (2000), Ettema *et al.* (1999) and Chicheley. The curves are redrawn from the exponentials fitted to the datasets from Figures 2b and 4 a & b and the different absolute amounts of denitrification were scaled to the maximum value measured.

#### 4.1.4 Conclusion

The result from a stepwise regression showed that the best model for denitrification in the riparian ecosystem studied in this thesis included water-filled pore space and soil  $\text{NO}_3^-$  as the main explanatory variables and that WFPS explained most of the variability. Denitrification rates measured in an intact riparian site at Chicheley were exponentially correlated to the water-filled pore space of the soil. This result is in agreement with studies using soil cores incubated either in the field or the lab from a wider variety of different ecosystems. All studies showed similar threshold values of soil moisture (60-80% WFPS; 20-40% gravimetric moisture), but different absolute rates of denitrification. The absolute rates were related to the soil  $\text{NO}_3^-$  concentration – fertilised sites showed denitrification rates approximately 20 times higher than unfertilised. This suggests that denitrification rates can be relatively simply modelled by using a general exponential relationship between denitrification rate and water-filled pore space (or volumetric/gravimetric water content) multiplied by a constant value depending upon the nitrogen status of the site.

At Chicheley and in several other studies, high rates of denitrification were possible at low temperature if WFPS was high. In contrast, if temperature is high but WFPS is low, denitrification rates were low or negligible. This confirms the argument that, within the ranges of temperature common in temperate environments, temperature is less important than moisture for initiating denitrification. This study also showed that for soil volumetric water contents below 35%, gaseous N was emitted as 50%  $\text{N}_2\text{O}$  and 50%  $\text{N}_2$  at the lower, intermediate and upper levels above the stream surface. Above this moisture level, the partition was 80%  $\text{N}_2$  and 20%  $\text{N}_2\text{O}$ , 90%  $\text{N}_2$  and 10%  $\text{N}_2\text{O}$  and 60%  $\text{N}_2$  and 40%  $\text{N}_2\text{O}$ , at the lower, intermediate and upper level respectively. However, there was high variability on these estimates. Based on this study, it seems that the denitrification equations in INCA should be modified so that the relationship between

denitrification and soil moisture has an exponential form. It is also recommended that the direct soil moisture measurements be substituted for the normalised soil moisture deficit currently used in INCA and that the soil moisture threshold be set at approximately 70%. This would constrain the model based on observed data.

## **4.2 Denitrifying Enzyme Activity experiment**

### **4.2.1 Introduction**

Denitrification rates under natural conditions are influenced by the size and potential activity of the existing population of soil denitrifying organisms and a range of environmental factors (Firestone, 1982). One of the major approaches to assess the potential activity of the denitrifier population is the short-term denitrification enzyme activity (DEA) assay (Chapter 2, section 2.5). The DEA assay has been developed for measuring the activity of the denitrifier population in sampled soils rather than *in situ* (Smith and Tiedje, 1979; Tiedje, 1982). Measurement of DEA is usually conducted with non-limiting substrate and under anaerobic conditions. To reflect the existing denitrifying activity in the soil, however, the assay can only be performed for a few hours. The assay is recommended for use in denitrification studies, since the measured DEA can reflect the recent environmental history of the site and offer the possibility of estimating field denitrification rates (Tiedje *et al.*, 1989).

The incubation time for the assay has to be short enough to avoid measuring denitrification from new organisms, but long enough to allow the products of denitrification to be measured accurately. The use of an antibiotic, chloramphenicol, inhibits protein synthesis and extends the measurable period for existing activity (Tiedje *et al.*, 1989). However, chloramphenicol may have side-effects on the denitrification

process (Smith and Tiedje, 1979) and it should not be used if the period of DEA measurement is more than 3 hours.

#### **4.2.2 Materials and methods**

##### ***Soil sample preparation***

Soil samples were taken from the two riparian sites at Chicheley, Chicheley East and North (Chapter 2, section 2.3). A first trial was done to establish the exact location of the high denitrification activity at our sites. Samples were taken from different soil depths (0-10cm, 10-20 cm and > 20 cm) on each level of the slope at both sites. The results showed the highest activity occurred in soils from the 0-10 cm layer. The DEA assay was thereafter conducted on soil samples from this layer. For each level of the slope, several soil samples were taken and bulked together to obtain one sample per level. Soils were collected during summer and autumn 2002 and spring 2003. The field-moist samples were sieved in the laboratory immediately after sampling. The visible roots were removed and the samples were riffled several times to ensure homogeneity. Sieved soil samples were stored overnight in bags at room temperature and assessed for DEA the next morning.

##### ***Procedure***

The assay technique involved anaerobic incubation of soil samples in the presence of acetylene to prevent conversion of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Yoshinari *et al.*, 1977).  $\text{N}_2\text{O}$  is the sole gaseous product of denitrification in soils incubated in atmospheres containing 0.1-10% v/v  $\text{C}_2\text{H}_2$ , and the moles of  $\text{N}_2\text{O}$  produced (with  $\text{C}_2\text{H}_2$ ) are equal to the moles of  $\text{N}_2\text{O} + \text{N}_2$  (without  $\text{C}_2\text{H}_2$ ) (Yoshinari *et al.*, 1977). The method used to measure denitrification activity is described in Appendix III.



### *Analytical methods*

Periodically gas samples were collected from the flasks and transferred to evacuated 10 ml vials.  $\text{N}_2\text{O}$  was measured using an Ai Qualitek GC94 gas chromatograph equipped with an electron capture detector GC ECD (Appendix II). The denitrification activity was calculated as described in Appendix III.

The moisture contents of the soil immediately before each incubation experiment were measured. Duplicate samples of moist soil were dried at  $105^\circ\text{C}$  overnight to determine the gravimetric soil moisture.

### *4.2.3 Results and discussion*

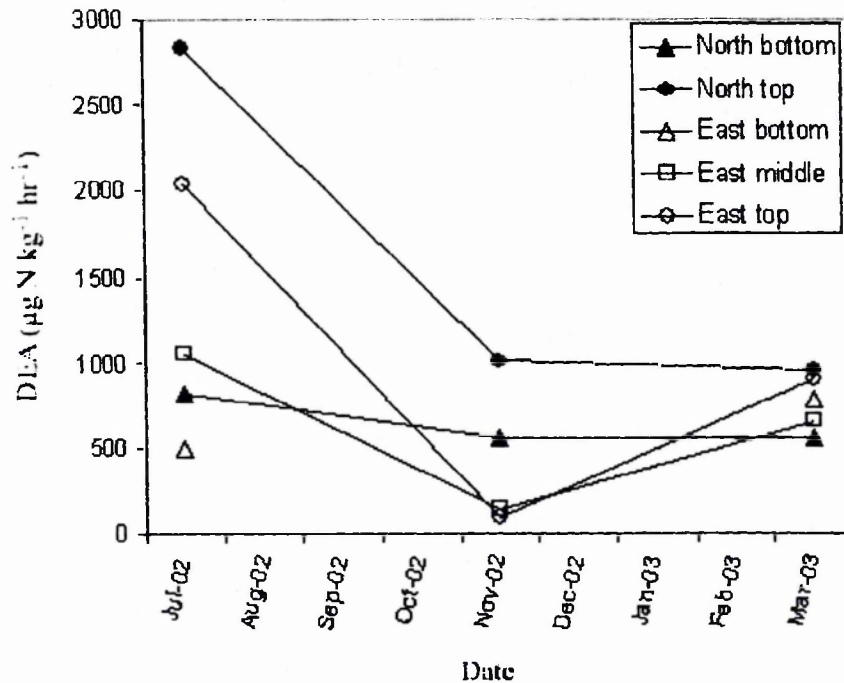
#### *Potential denitrification in soils*

The DEA results showed substantial differences among different soils from different locations across and within slopes. The lowest and highest DEA values were  $2.6 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ h}^{-1}$  and  $1827.8 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ h}^{-1}$ , and were measured at the upper level of the slope at Chicheley East in March 2003 and the upper level of the slope at Chicheley North in July 2002, respectively. Substantial differences in DEA, reaching one order of magnitude were also found among soils from different locations within slopes in 2002. DEA from the same soils compared in time differed as well, but these differences were smaller, as can be seen from the relatively small standard deviations in most cases except for DEA measured in March 2003 (Table 4.2). In November 2002, no DEA was carried out on soil samples from the Chicheley East lower slope location since the site had flooded and the lower position of the slope was still under water at the time of sampling.

**Table 4.2:** Denitrifying enzyme activity (DEA) and moisture content (% mass/mass) in soils sampled in July and November 2002 and March 2003. Means with standard deviations in parentheses. ND: Not Determined.

Site	DEA ( $\mu\text{g N}_2\text{O-N kg}^{-1} \text{ h}^{-1}$ )			Moisture (%, mass/mass)		
	31/07/2002	14/11/2002	26/03/2003	31/07/2002	14/11/2002	26/03/2003
Ch North upper	1798.6 (38.5), n=3	642.9 (168.0), n=3	604.5 (358.8), n=5	27.6	30.8	18.2
Ch North lower	520.0 (89.2), n=3	357.4 (28.8), n=3	356.3 (170.1), n=5	24.4	30.2	19.3
Ch East upper	1297.4 (76.7), n=3	59.5 (29.7), n=3	575.6 (459.9), n=4	32.1	30.8	31.5
Ch East intermediate	668.8 (65.1), n=3	86.7 (10.5), n=3	421.2 (134.0), n=4	23.0	30.5	28.4
Ch East lower	313.7 (34.1), n=3	ND	500.6 (639.2), n=4	18.6	ND	21.9
Range	279.5 - 1827.8	25.3 - 780.8	2.6 - 1447.9			

DEA showed the same seasonal pattern at both sites and on each different location of the slope. DEA was highest in summer 2002 at every location of the slope at both Chicheley North and Chicheley East (Fig 4.5). DEA decreased in November 2002 and either increased or stayed stable in March 2003. In contrast, denitrification rates were very low in summer 2002, stayed stable or slightly decreased in autumn 2002 and increased in March 2003. This is an indication that, even when field conditions are limiting denitrification, the soil denitrifier population and the potential for denitrification in the soil persist. Our results also showed that the DEA was higher in the upper soils than in the lower soils in July 2002, November 2002 and March 2003 at Chicheley North and July 2002 and March 2003 at Chicheley East. This pattern follows the soil moisture pattern and is discussed in the next section.



**Figure 4.5:** Seasonal variation of Denitrifying Enzyme Activity (DEA,  $\mu\text{g N kg}^{-1} \text{ hr}^{-1}$ ) in time at the different locations of the slope at Chicheley North and Chicheley East. One measurement is missing at the lower location of the slope at Chicheley East.

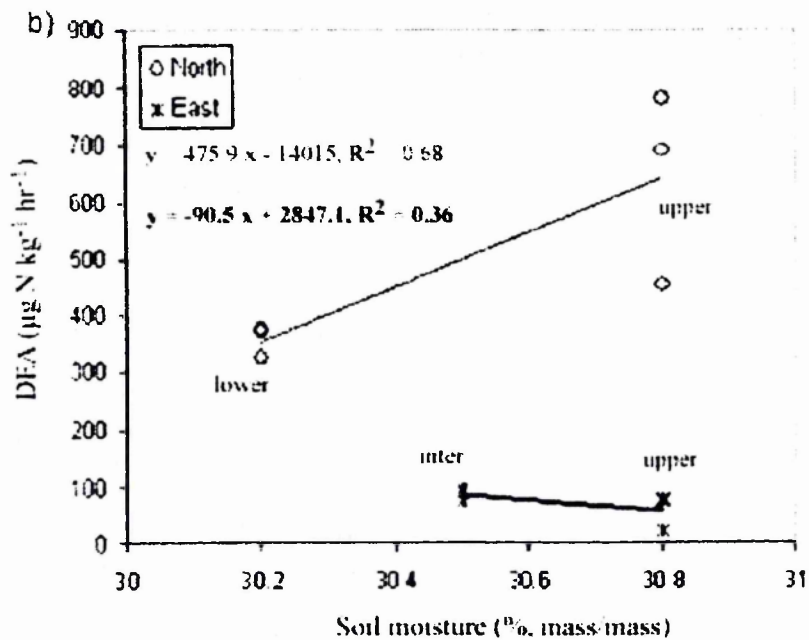
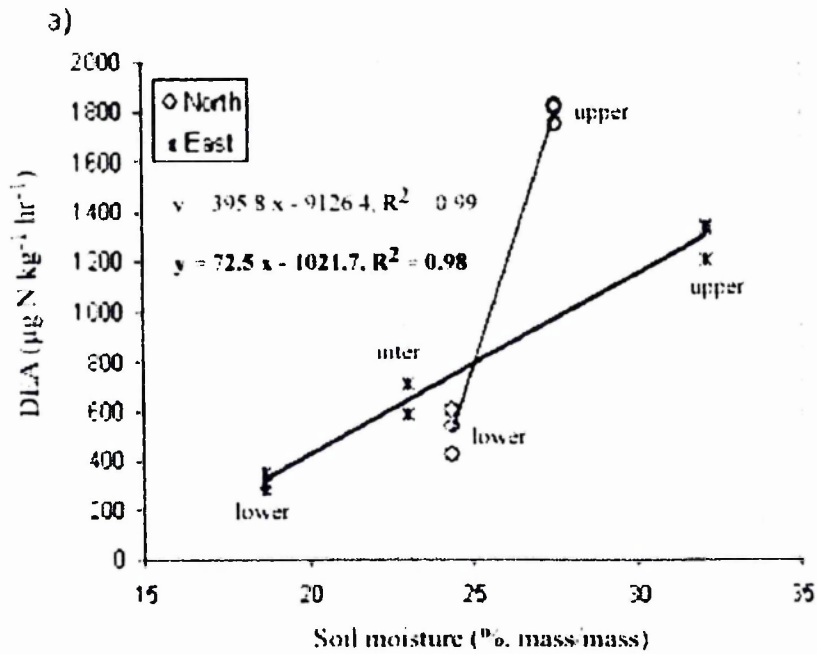
No relationship was found between the annual field denitrification rates and DEA. Groffman and Tiedje (1989b), in a study of denitrification and environmental parameters in north temperate forest soils, concluded that only a small percentage of denitrification enzymes are active at any one time in a soil and showed that the occurrence of denitrifying bacteria in any given habitat is determined more by the ability of these bacteria to compete as heterotrophs rather than by their ability to denitrify. This could explain the differences we observed on different sampling occasions. Thus both our study and that of Groffman and Tiedje (1989b) suggest that DEA is better interpreted as an estimate of the biomass of denitrifying bacteria in soil rather than as an index of actual denitrification rates.

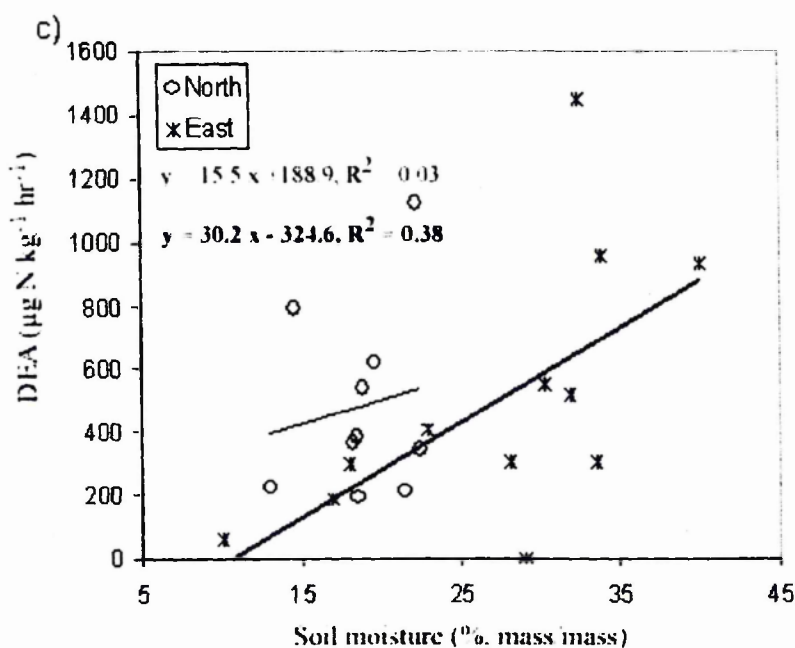
### *Relationships between DEA measurements and soil properties*

There was no statistically significant relationship between DEA and soil textures (% sand, silt and clay). In contrast, Groffman *et al.* (1992) reported a statistically significant relationship between DEA and soil texture in 22 north temperate forest soils.

The DEA measurements were carried out on triplicate bulk soil samples for each location of the slope at both sites on all occasions except in March 2003, when DEA was carried out on individual soil samples taken from each of the 22 flux chamber locations (10 soil samples for Chicheley North and 12 soil samples for Chicheley East; see chapter 2, section 3). In this study, the possible relationship between DEA and soil moisture was examined. The data showed that the results varied according to the season. DEA was strongly positively correlated with the soil moisture at both sites in July 2002 (Fig 4.6a). In a study of the control of denitrification enzyme activity in a streamside soil, Ambus (1993) also found that DEA in the surface soil was significantly related to the soil water content (mass/mass). In July 2002 and less significantly in November 2002, the soil moisture at our sites decreased from the upper location of the slope to the lower location due to differences in soil texture as discussed in Chapter 3, section 3.4.2. On these occasions and in March 2003 at Chicheley East, DEA was higher at the upper locations of the slopes, following the soil moisture. In contrast, denitrification fluxes of  $N_2 + N_2O$  were generally higher at the lower location of the slope. This could be explained by the possibility that the lower location of the slope is the site of substantial denitrification of streamwater moving from Chicheley Brook into the riparian zone. However, further investigation is needed to test this hypothesis. In our study, there was a weaker, but still significant positive relationship between soil moisture and DEA at Chicheley North in November 2002 but not in March 2003. At Chicheley East DEA was weakly negatively correlated to soil moisture in November 2002 (Fig 4.6b). The data on this particular occasion are difficult to interpret since no measurements were done on soils from the lower location of the slope because it was

flooded. In March 2003 (Fig 4.6c), there was a weak positive relationship ( $r^2 = 0.38$ ) between soil moisture and DEA for the Chicheley East data.





**Figure 4.6:** Relationship between DEA ( $\mu\text{g N kg}^{-1} \text{ hr}^{-1}$ ) and soil moisture at Chicheley North and Chicheley East in a) July 2002, b) November 2002 and c) March 2003. Linear trends, equations and correlation coefficients for Chicheley North data (light) and Chicheley East data (bold) are on each plot.

#### 4.2.4 Conclusion

This study showed that denitrifying enzyme activity (DEA) in riparian soils varies according to the season. Substantial differences among sites as well as differences in time were observed for the soils studied. The same seasonal pattern was found in all soils, with highest DEA rates measured in July 2002 and the lowest measured in November 2002. There was no relationship between DEA and annual field denitrification rates, perhaps because DEA indicates overall rates of denitrification which were not constrained by the field denitrification measurements. Therefore DEA could not be considered as an index of actual denitrification rates. In general, DEA was positively related to soil moisture.

## **Chapter Five**

### **Modelling denitrification in riparian ecosystems: calibration and application of the INCA model**

#### **5.1 Introduction**

The purpose of the denitrification-nitrous oxide study within the INCA project was to refine the equation describing denitrification in INCA as it is important to the development of a generic version of the model applicable to a wide range of ecosystems and catchments. The INCA model was described in Chapter 1, section 5, which gives details of the equations used up to now to simulate denitrification. More details of the hydrological and biochemical process equations used to simulate nitrogen transportation and transport through terrestrial systems into rivers can be found in Whitehead *et al.* (1998a).

In this chapter, a new version of the INCA model, specifically implemented to evaluate the nitrogen balance in riparian ecosystems, was calibrated and applied to our Chicheley riparian study. The results from the field denitrification study undertaken at

Chicheley (Chapter 4) were used to calibrate the model and compare the outputs of the model simulation of denitrification rates to those observed *in situ*.

Since the site is within the extensively modelled Great Ouse river basin in eastern England an existing INCA parameter file for this catchment was used. It was then necessary to obtain the data covering the field experimental period. The input data files needed include time step, input hydrological data (Soil Moisture Deficit, Hydrologically Effective Rainfall, Air Temperature and Actual Precipitation), and effluent time series containing flow ( $\text{m}^3 \text{s}^{-1}$ ), nitrate ( $\text{mg l}^{-1}$ ) and ammonium ( $\text{mg l}^{-1}$ ) for each reach in the system (Table 5.1). Once the data were collected, the input files were set up. The model was calibrated using the Bedford Ouse parameter file and the input data files set up for the same period as the experimental period. The parameter file was then modified to obtain satisfying calibration's results, and this modified parameter file used to run the model for comparison with the field experimental results.



**Table 5.1:** Summary of data used in INCA modelling of the Bedford Ouse.

<i>Data</i>	<i>Description</i>	<i>Source of data</i>	<i>Reference</i>
Streamwater NO <sub>3</sub> -N and NH <sub>4</sub> -N concentrations	Samples from 13 sites along the main stem of the Bedford Ouse river. Monthly sampling for 2001 – 2003	Environment Agency	
Effluent NO <sub>3</sub> -N and NH <sub>4</sub> -N concentrations	Samples from 8 STWs along the main stem of the Bedford Ouse river. Monthly sampling for 2001 – 2003	Environment Agency	
Effluent flows	Annual mean flows derived from Population Equivalent data for STWs	Environment Agency	
River flows	Mean daily flows for four gauging stations on the main stem of the Bedford Ouse (2001 – 2003)	National Water Archive	Marsh and Lees, 2003
MORECS rainfall, temperature and soil moisture deficit	Derived daily time series	Meteorological Office	Hough <i>et al.</i> , 1997
Base Flow Index	Derived for each flow gauging station and extrapolated to ungauged river reaches	Centre for Ecology and Hydrology, Wallingford	Gustard <i>et al.</i> , 1992
Fertiliser application rates	Average annual rates of fertiliser applications	British Survey of Fertiliser Practice	The Stationery Office, 1997

## 5.2 The Bedford Ouse catchment

The River Great Ouse in eastern England (Fig 5.1) is a lowland agricultural catchment with an area of 8380 km<sup>2</sup>, including most of Cambridgeshire and Bedfordshire and part of seven other counties. Two major river systems, the Bedford Ouse and the Ely Ouse, converge at Denver in Norfolk, releasing an average of 38.5 m<sup>3</sup> s<sup>-1</sup> of freshwater into the Great Ouse estuary and The Wash at King's Lynn (Whitehead *et al.*, 1998b). The catchment has a resident population of approximately 1.6 million which results in effluent discharges from over 500 sewage and industrial treatment plants (e.g. Milton Keynes, Cambridge, Bedford, King's Lynn).

The river and its tributaries are used for public water supply from six separate intakes. In addition, large quantities of water are transferred into neighbouring catchments for potable water supplies using the Ely Ouse-Essex water transfer scheme.

Our study is concerned with the Bedford Ouse river system which rises to the north of Brackley and flows north-eastwards across the Oolitic and Cornbrash limestones to Newport Pagnell. Below Newport Pagnell the geology becomes impermeable clays (Amphill, Kimmeridge and Oxford) which persist until Oxford where the geology becomes dominated by chalk (Whitehead *et al.*, 1998b). The river continues to flow in a north-eastwards direction to Brownhill Staunch which forms the downstream boundary of the Bedford Ouse river system in our study. Land use in the Bedford Ouse catchment is strongly dominated by arable land but with large urban centres located at Milton Keynes and Bedford. Grazing land and fertilised grassland are also found in the upper reaches, as shown in the land use map in Fig 5.2.

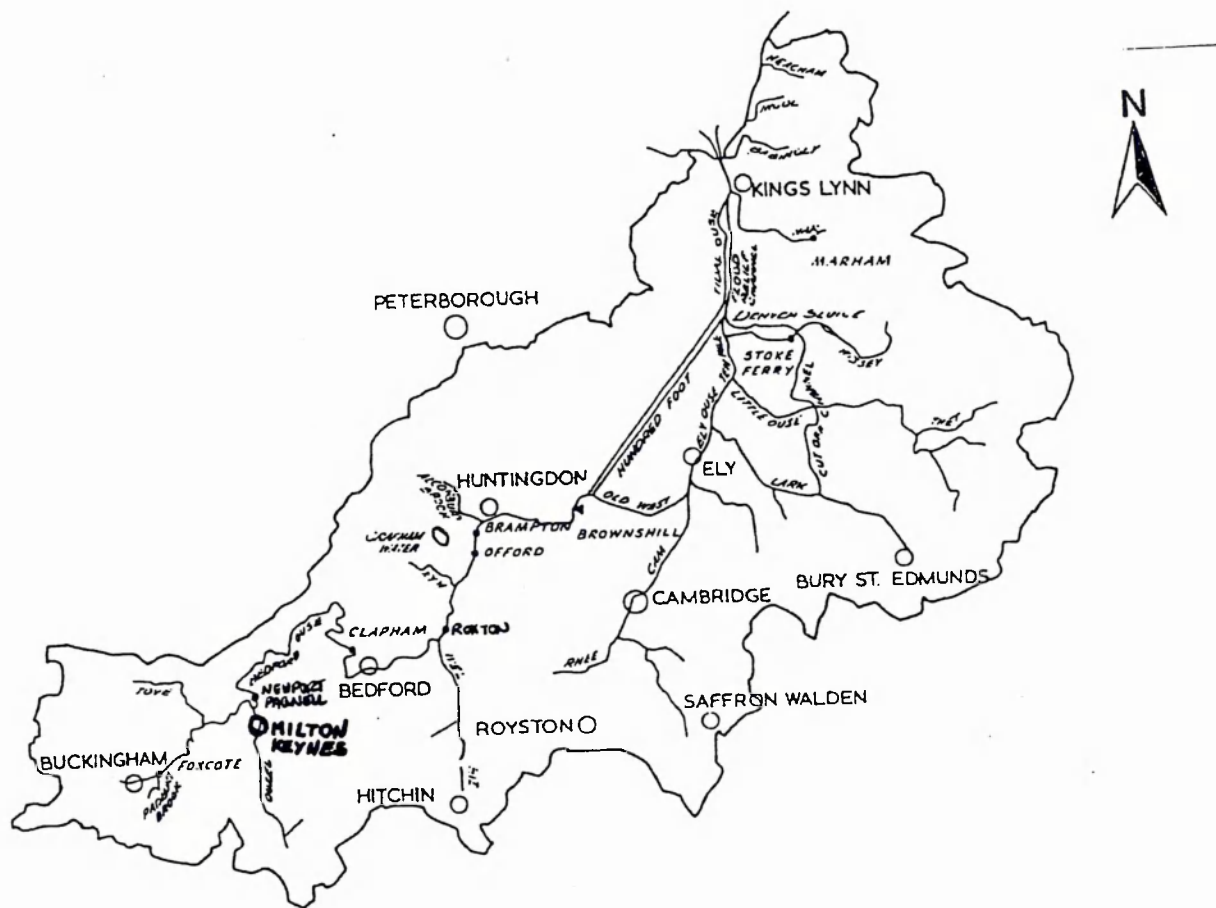
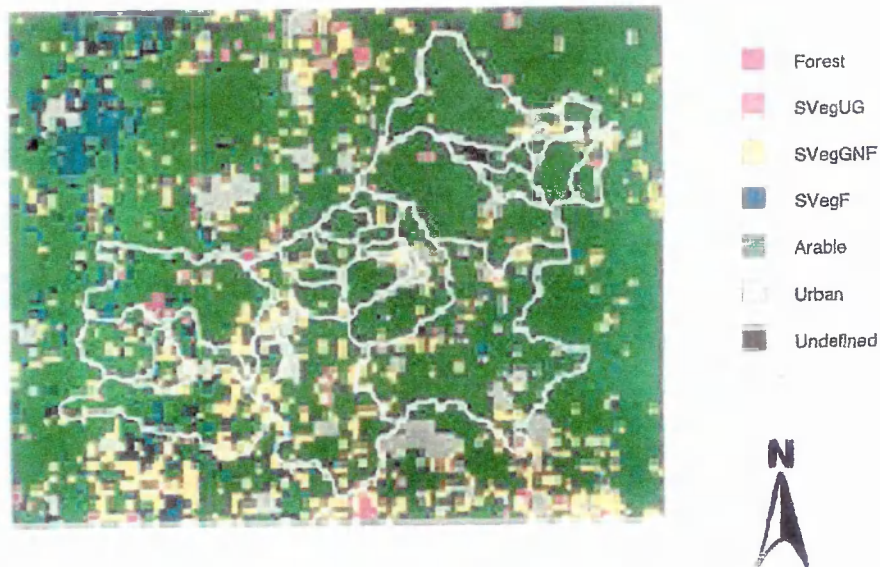


Figure 5.1: Map of the River Great Ouse system, eastern England.



**Figure 5.2:** Land use map for Bedford Ouse area (Whitehead *et al.*, 1998b).

### 5.3 Model setup

The INCA model was designed to investigate the fate and distribution of nitrogen in the aquatic and terrestrial environment. There are five components to modelling nitrogen in catchments using INCA (see Chapter 1, section 5): a GIS interface, the nitrogen input model, the hydrological model, the catchment nitrogen process model and the river nitrogen process model. In order to run INCA it is necessary to provide two data files: a catchment description and process parameter file, and a hydrological daily time series file. The catchment and parameter file consists of the following information:

- 1- *reach structure, land class percentages and base flow characteristics.*
- 2- *deposition data* (atmospheric wet and dry nitrogen deposition in the sub-catchments).
- 3- *land process parameters*, which are associated with each N processes and control the dynamic response.
- 4- *river data and parameters*: the river data consists of information on reach lengths, velocity-flow information and sewage inputs; initial nitrate and ammonium concentrations have to be specified for the water draining the soil zone and the groundwater zone; the model also requires the user to specify the parameters that control nitrification and denitrification processes occurring in the river as these processes are particularly important during low flow summer conditions when residence times and temperatures are high.
- 5- *input time series data file*: hydrological input data are required to drive the hydrological component of the INCA model. They consist of daily time series of hydrologically effective rainfall (HER, rainfall which penetrates the ground after allowing for evapotranspiration and interception losses), soil moisture deficit and temperature (Fig 5.3).
- 6- *observed data file*: the final data set consists of observed flow and water quality for the river at any reach boundary.

To model nitrogen in the Bedford Ouse using INCA, the main stem of the Bedford Ouse was sub-divided into 26 reaches of less than 20 km in length (Table 5.2). The reach boundaries were derived from an analysis of the digital terrain model (DTM) information making use of catchment boundary algorithms developed at the Institute of Hydrology. Similar algorithms are available in GIS systems such as ARC-INFO. Reach boundaries were designed to coincide with key factors controlling flow and water quality such as sub-catchment tributary inputs, effluent discharges, and the location of flow gauging stations and water quality monitoring sites. The location of gauging stations and water

quality monitoring sites at reach boundaries facilitates comparison of model simulations with observed flow and chemical concentration data at specified sites along the river. Table 5.2 shows the reach structure selected for the Bedford Ouse together with information on reach length, sub-catchment area and the land use percentages obtained from the GIS-INCA interface. The areas given in Table 5.2 were also derived from the DTM analysis via Institute of Hydrology algorithms.

Velocity-flow information is required to estimate residence times of water within each river reach. Velocity-discharge information for the Bedford Ouse from a set of tracer experiments conducted by Whitehead *et al.* (1986) led to the following relationship:

$$v = 0.046 Q^{0.64}$$

where  $v$  is the mean flow velocity in the reach ( $\text{m}^3 \text{s}^{-1}$ ) and  $Q$  represents the discharge ( $\text{m}^3 \text{s}^{-1}$ ).

In INCA, the baseflow index (Gustard *et al.*, 1987) is used to partition the water moving between the soil water and ground water reservoirs (Wade *et al.*, 2002). The baseflow index is a measure of the proportion of river runoff which is derived from stored sources. Table 5.3 shows the base flow index (BFI) for the 26 reaches along the Bedford Ouse used in INCA for the period from 2001 to 2003. The base-flow index information can be obtained from the hydrological year-books (Institute of Hydrology, 1991) or from the application of time-series modelling technique such as IHACRES (Jakeman *et al.*, 1990). Where reaches contain no flow gauging monitoring stations, BFIs were determined by extrapolating a known BFI downstream until a monitoring station is encountered and the new BFI used.

**Table 5.2: Reach and land use information for the Bedford Ouse.**

Reach number	Reach length (m)	Area km <sup>2</sup>	Forest (%)	SVegUG (%)	SVegGNF (%)	SVegF (%)	Arable (%)	Urban (%)
1	17750	102	3	0	11	10	75	1
2	10000	51	0	0	20	15	59	6
3	1250	238	1	0	17	20	62	0
4	6500	56	4	0	12	13	71	0
5	6500	290	4	0	7	7	81	0
6	6500	50	0	0	24	12	42	22
7	6000	384	2	0	16	3	71	8
8	9250	74	1	0	10	0	88	1
9	5500	37	0	0	10	3	84	3
10	8000	33	0	0	9	0	91	0
11	7000	31	0	0	0	0	100	0
12	7000	30	0	0	10	0	90	0
13	7000	15	0	0	0	0	100	0
14	7000	14	0	0	0	0	100	0
15	10000	66	0	0	9	0	80	11
16	3750	12	0	0	17	0	0	83
17	4500	147	0	0	7	1	85	7
18	6750	577	1	0	7	1	83	8
19	9500	325	1	0	5	1	91	2
20	6250	34	0	0	3	0	94	3
21	5750	36	0	0	3	0	94	3
22	4000	13	0	0	8	0	77	15
23	7000	251	0	0	6	3	88	3
24	2750	91	0	0	7	1	91	1
25	2500	38	0	5	11	0	84	0
26	2000	1	0	0	0	0	100	0

**Table 5.3:** Base flow index, effluent discharge and effluent NH<sub>4</sub> and NO<sub>3</sub> concentrations for the Bedford Ouse.

Reach number	Reach name	BFI	Effluent NH <sub>4</sub> -N concentration (mg-N L <sup>-1</sup> )	Effluent discharge (m <sup>3</sup> s <sup>-1</sup> )	Effluent NO <sub>3</sub> -N concentration (mg-N L <sup>-1</sup> )
1	one	0.51	0	0.023	0.3
2	two	0.51	11	0.023	0.7
3	three	0.51	0	0	0
4	Thornborough	0.51	0	0	0
5	five	0.51	0	0	0
6	six	0.51	0	0	0
7	seven	0.51	0	0	0
8	Newport Pagnell	0.51	7.3	0.023	0.9
9	nine	0.51	0	0	0
10	ten	0.51	0	0	0
11	Harold Mill	0.51	0	0	0
12	twelve	0.51	0	0	0
13	thirteen	0.51	0	0	0
14	fourteen	0.51	0	0	0
15	Bedford	0.51	0	0	0
16	sixteen	0.48	0	0	0
17	seventeen	0.48	10.6	0.023	1.7
18	Roxton	0.48	0	0	0
19	nineteen	0.48	0	0	0
20	Offord	0.48	16	0.023	4.5
21	twenty-one	0.48	0	0	0
22	twenty-two	0.48	19.9	0.023	0.4
23	twenty-three	0.48	23.9	0.023	1.3
24	twenty-four	0.48	0	0	0
25	twenty-five	0.48	0	0	0
26	Brownhill Stauch	0.48	21.5	0.023	0.5

## WATER CHEMISTRY DATA

The water chemistry data were obtained from the Environment Agency (EA), Anglian Region. The water quality sampling took place on a monthly basis at 13 sites on the Bedford Ouse from 2001 to 2003. These 13 sites included Newport Pagnell (Reach 8), Roxton (Reach 18), Offord (Reach 20) and Brownhill Stauch (Reach 26) which are used here. The Environment Agency also collects effluent chemistry and flow data, which provide another important input to the INCA model. This provided a dataset for the Bedford Ouse catchment spanning from 2001 to 2003. The 13 EA sites provided the NO<sub>3</sub><sup>-</sup> concentration data for INCA.

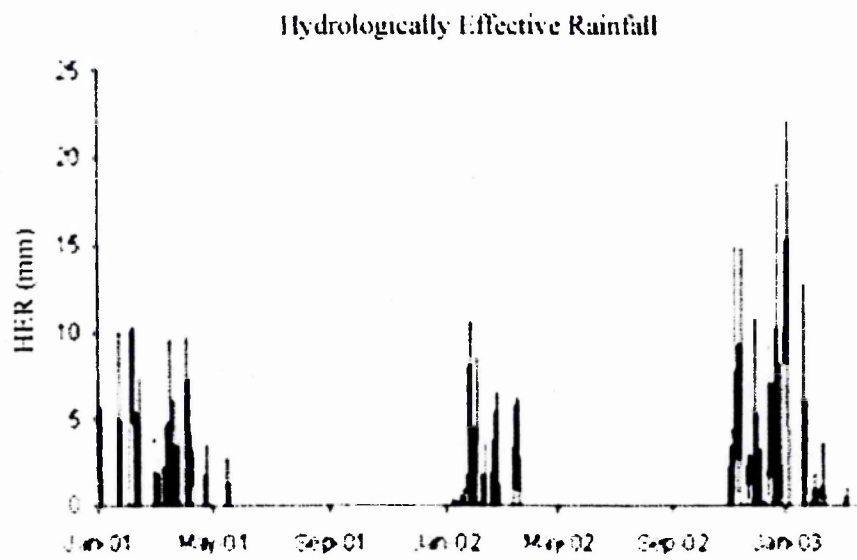
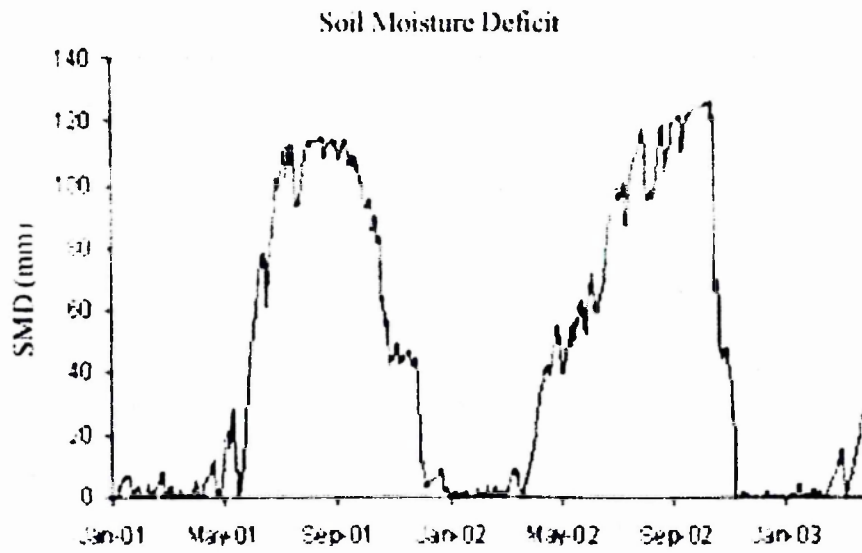


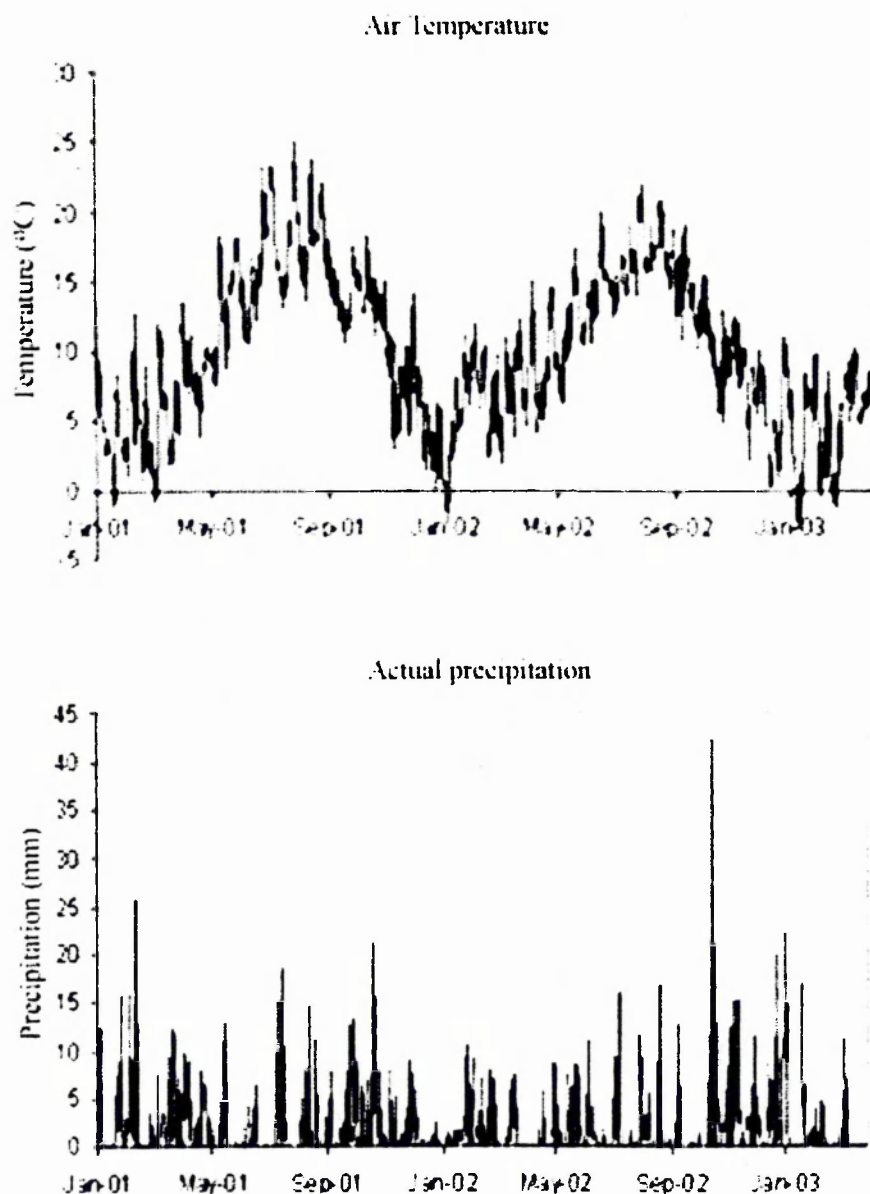
## DEPOSITION CHEMISTRY

Nitrogen inputs from atmospheric deposition were estimated using the MATADOR-N model (Model of Atmospheric Transport And Deposition Of Reaching Nitrogen, Rodgers, 1993; RGAR, 1997). This model provides an estimate of long-range transport and wet and dry deposition of oxidised and reduced N ( $\text{NO}_x$  and  $\text{NH}_y$ ). Details of calculation methods are provided elsewhere (Whitehead *et al.*, 1998a; Wade *et al.*, 2001). Using MATADOR-N, the mean annual wet and dry deposition over the Bedford Ouse catchment was estimated as  $6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  for both  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ , which is a total of  $12 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ .

## HYDROLOGICAL DATA

Four flow gauging stations on the main stem of the Bedford Ouse supplied observed mean daily river flows. The hydrological input data demonstrate a high degree of variability in the conditions experienced over the 27-month study period (Fig. 5.3). There were relatively high soil moisture deficits between May 2001 and January 2002 and April and November 2002, as well as two prolonged periods with no HER between May 2001 and January 2002 and March to October 2002. The hydrological input data were supplied by the UK Meteorological Office, based on meteorological observations and output from the MORECS soil moisture and evaporation accounting model. For the Bedford Ouse river, the baseflow index ranges from 0.48 to 0.51 (Institute of Hydrology, 1998).





**Figure 5.3:** MORECS data for the Bedford Ouse catchment: hydrological time series input for INCA (Soil Moisture Deficit (SMD), Hydrologically Effective Rainfall (HER), air temperature and actual precipitation from 2001 to 2003).

## CATCHMENT LAND USE

Sub-catchment areas draining to each of the 26 river reaches were defined using the Institute of Hydrology Digital Terrain Model (IHDTM) within a Geographical Information System (GIS; ARC/INFO). Within each of the sub-catchment areas, the land use characteristics were derived from the Institute of Terrestrial Ecology (ITE, now CEH) Land Cover data (Fuller, 1993). The twenty five ITE land cover classes were then

grouped into six categories, as defined by Whitehead *et al.* (1998a): (i) forest, (ii) short vegetation ungrazed, (iii) short vegetation grazed not fertilised (unimproved grassland), (iv) short vegetation grazed and fertilised (improved vegetation), (v) arable, (vi) urban. Details of the sub-catchment areas draining to each of the Bedford Ouse river reaches and percentage cover of each land use type within each sub-catchment are shown in Table 5.2.

#### LAND MANAGEMENT AND PLANT/CROP GROWTH PERIODS

Typical fertiliser application rates to arable and improved grassland were estimated as 53 kg-N ha<sup>-1</sup> yr<sup>-1</sup> for both NO<sub>3</sub>-N and NH<sub>4</sub>-N applications to improved grassland and 97 kg-N ha<sup>-1</sup> yr<sup>-1</sup> for both NO<sub>3</sub>-N and NH<sub>4</sub>-N applications to arable land. The timings of applications were estimated to run between 1<sup>st</sup> March and 1<sup>st</sup> September, based on local farming knowledge (Appendix XI). It was assumed that fertiliser input occurred evenly over the period of application and was predominantly applied in the form of ammonium nitrate. The main plant growing season was estimated to begin on 1<sup>st</sup> March and end on 31<sup>st</sup> October, with the exception of arable land, where the growing season was estimated to end at harvest time (7<sup>th</sup> July).

### 5.4 Model calibration and Bedford Ouse simulation

The model was calibrated using data for the years 2001 to 2003. Model calibration was undertaken in three steps:

1. *Hydrology.* Simulation of nitrogen concentrations and loads in both catchment and stream components is dependent on water volumes and the routing of water through the soil, groundwater and river reaches. Therefore, it is important to simulate hydrology accurately. Parameters relating to the flow-velocity

relationship were set according to experimental tracer observations. Constants defining the residence times of water in the soil and groundwater reservoirs were determined through calibration, until the simulated mean daily flows closely matched the observed mean daily flows for the 2001-2003 period.

2. *Initial conditions.* Having set the fertiliser applications and plant growth periods according to local land management practices, the second step in the calibration procedure involved adjusting initial  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations in the soil, groundwater and in-stream components, so that the simulated flow and initial  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations in the first few days of the model run matched observed in-stream concentrations. By running the model to simulate a three-year period with a daily time step, the influence of initial conditions on model results was minimised.
3. *Process rates.* Parameters relating to soil nitrogen processes (rates of  $\text{NH}_4\text{-N}$  immobilisation,  $\text{NO}_3\text{-N}$  denitrification,  $\text{NH}_4\text{-N}$  nitrification,  $\text{NH}_4\text{-N}$  mineralization, and plant  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  uptake) and in-stream rates of denitrification and  $\text{NH}_4\text{-N}$  nitrification were adjusted so that (i) the simulated annual fluxes for catchment processes and annual leaching loads were largely within expected ranges of published data for relevant land use types (Table 5.4), and (ii) the simulated daily  $\text{NO}_3\text{-N}$  concentrations matched observed daily  $\text{NO}_3\text{-N}$  values as closely as possible during 2001-2003. The calibrated parameter values are shown in Appendix XI.

The loads derived from these values for each process can be compared with field measurements and provide a further means of calibrating the model in addition to matching the observed flows and streamwater  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations.

**Table 5.4:** Catchment process loads: comparing measured values ( $\text{kg-N ha}^{-1} \text{ yr}^{-1}$ ) within the published literature (from Battison, 2000; amended from Whitehead *et al.*, 1998a) with simulated mean annual fluxes ( $\text{kg-N ha}^{-1} \text{ yr}^{-1}$ ) during 2001 to 2003 in the Bedford Ouse catchment.

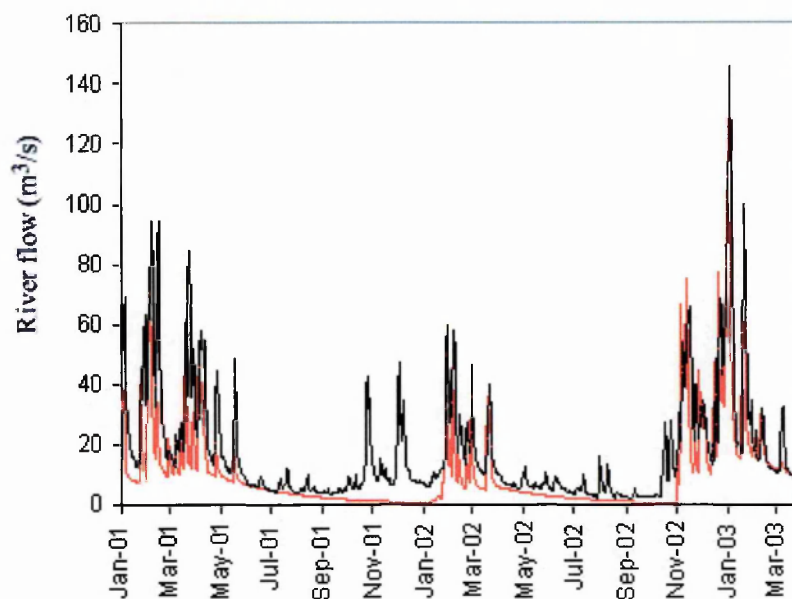
Land use / Process	Measured value or range in values	Simulated value for Bedford Ouse
<i>(1) NO<sub>3</sub>-N uptake</i>		
Forest	<5.1 – 153	41
Ungrazed short vegetation	42	46
Unimproved grassland	30 – 162	43
Improved grassland	105	112
Arable	95 – 200	128
<i>(2) Denitrification</i>		
Forest	<0.01 – 4	2.5
Ungrazed short vegetation	1 – 15	3
Unimproved grassland	1 – 4.4	2.6
Improved grassland	19	12
Arable	10 – 60	15
<i>(3) Nitrification</i>		
Forest	1 – 35	2.6
Ungrazed short vegetation	3 – 54	21
Unimproved grassland	3 – 54	8.5
Arable	10 – 50	81
<i>(4) Mineralisation</i>		
Forest	10 – 292	9
Ungrazed short vegetation	20 – 60	9
Unimproved grassland	7 – 162	9
Improved grassland	40 – 50	18
Arable	30 – 171	18
<i>(5) Inorganic N leaching</i>		
Forest	<0 – 30	29
Ungrazed short vegetation	1.8 – 5.3	29
Unimproved grassland	1.8 – 5.3	28
Arable	15 – 100	66

## COMPARING OBSERVED AND SIMULATED STREAMFLOW HYDROGRAPHS

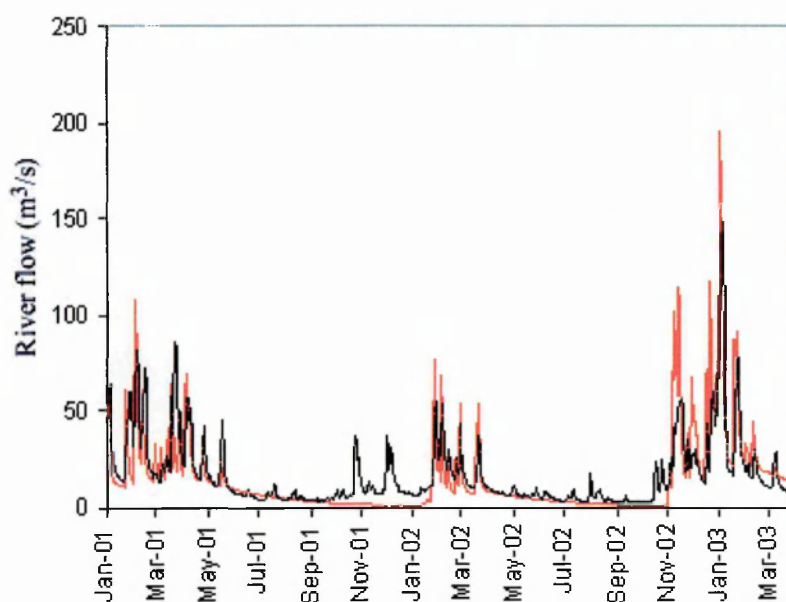
Observed mean daily river flows at Bedford, Roxton and Offord gauging stations from 2001 to 2003 and the corresponding simulated mean daily river flows, following calibration of the INCA model, are shown in Fig 5.4. The INCA hydrological calibration reproduces the dynamics of flow in the Bedford Ouse, although it tends to underestimate peak flows, especially at the upper site, Bedford. The model did not simulate the river flows between May 2001 and January 2002 and April 2002 and

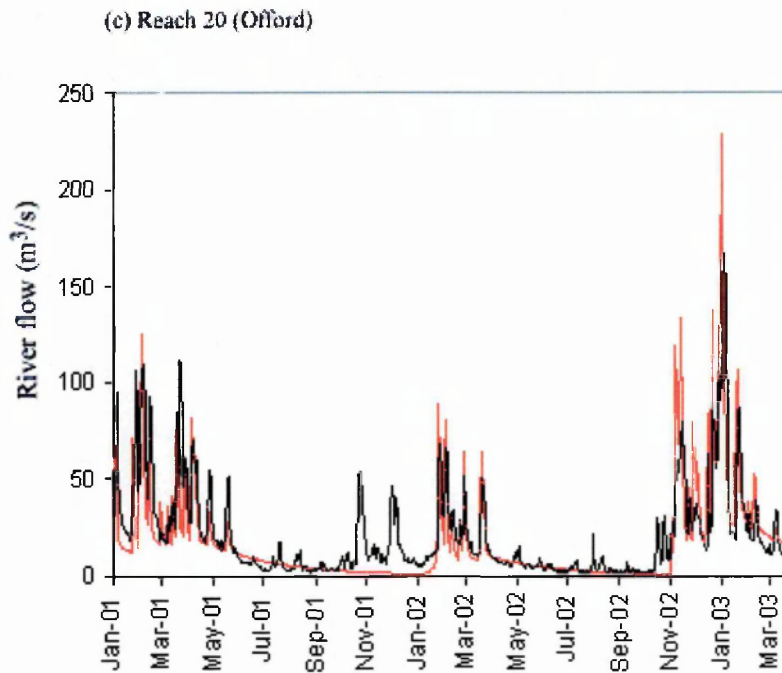
October 2002 due to the lack of hydrologically effective rainfall (HER) during these periods to generate flow. The simulations cannot be improved without improving the HER calculations.

(a) Reach 15 (Bedford)



(b) Reach 18 (Roxton)





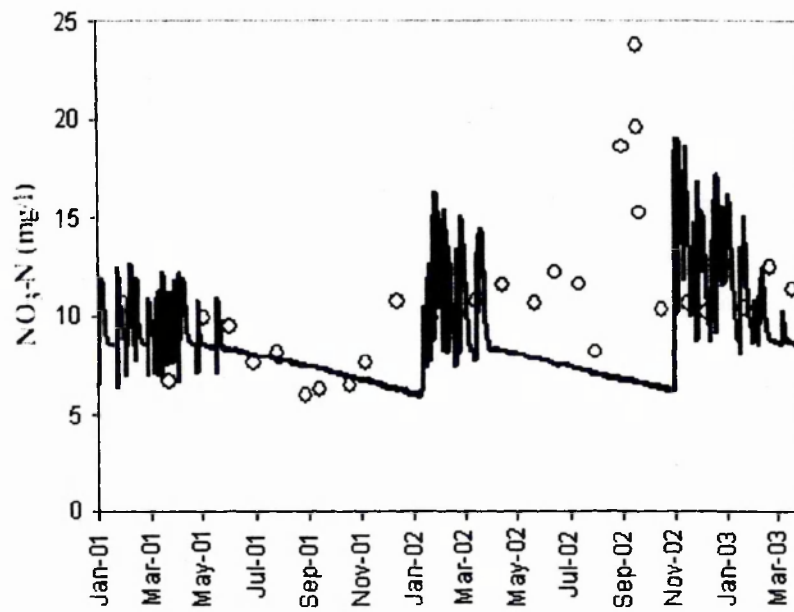
**Figure 5.4:** INCA calibration results for hydrology: observed and simulated river flows at Bedford (Reach 15), Roxton (Reach 18) and Offord (Reach 20) for 2001 to 2003. Observed flows are shown in black; simulated flows are shown in red.

## COMPARING OBSERVED AND SIMULATED NITRATE CONCENTRATIONS

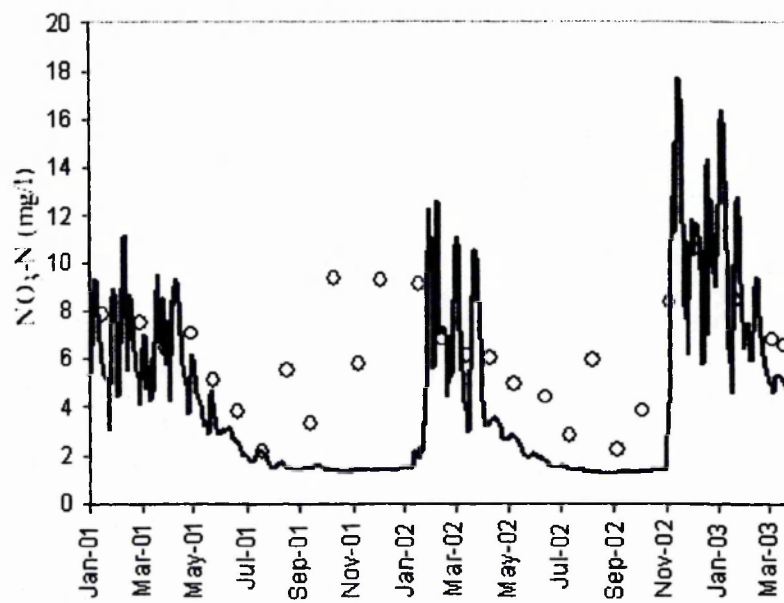
Examples of observed and simulated  $\text{NO}_3\text{-N}$  concentrations are shown in Fig 5.5 for sites in the upper catchment (Reach 2), middle catchment (Reach 17) and lower catchment (Reach 23). Figure 5.5 shows the dynamics of  $\text{NO}_3\text{-N}$  variability. These dynamics are represented adequately by model simulations from January through to May each year, especially in the lowest catchment. However, the  $\text{NO}_3\text{-N}$  variability is not simulated properly by the model for the rest of the year. The main problem seems to be an under-estimation of the nitrate concentrations during the periods of no-HER. As a result, the model does not generate the flow, and therefore simulate  $\text{NO}_3^-$  transport very well. This can only be improved by improving the general HER calculations, however, without any better way to estimate HER at present, the simulations are as good as can be.

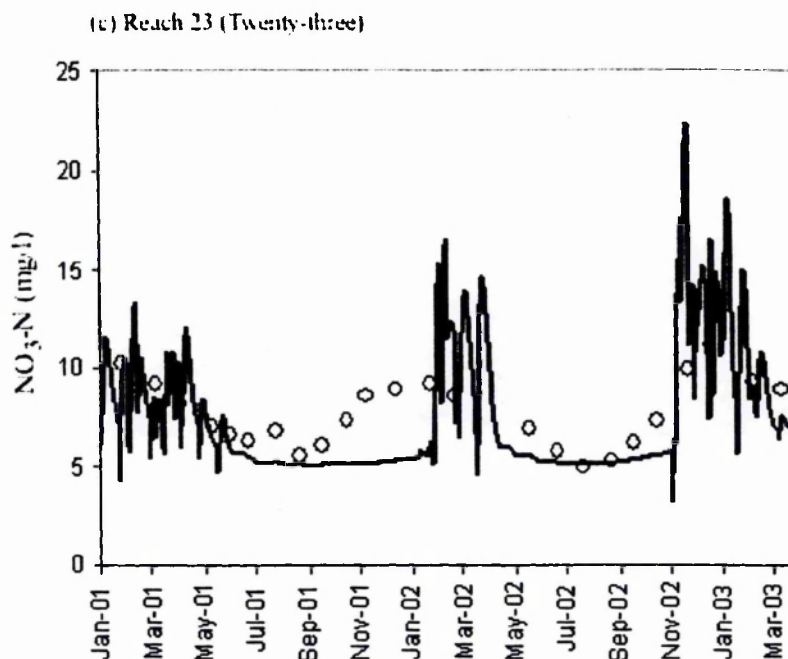


(a) Reach 2 (Two)



(b) Reach 17 (Seventeen)





**Figure 5.5:** INCA calibration results for nitrate: observed and simulated  $\text{NO}_3\text{-N}$  concentrations at Reach 2-upper, Reach 17-middle and Reach 23-lower for 2001 to 2003. Observed nitrate concentrations are shown as open circles; simulated nitrate concentrations are shown as a solid black line.

Both observed and simulated  $\text{NO}_3\text{-N}$  concentrations demonstrate well-defined seasonality (Fig 5.5), with lowest concentrations occurring during the summer and rising  $\text{NO}_3\text{-N}$  concentrations towards the end of autumn and winter, as soils wet up and nitrogen is flushed from the catchment. Typically, peak  $\text{NO}_3\text{-N}$  concentrations occur in January/February. The low summer concentrations reflect (a) vegetation uptake, probably the most important factor, (b) lower flows and thus reduced delivery of diffuse-source nitrogen from the catchment, and (c) higher water residence times within the river reaches, promoting greater in-stream denitrification.

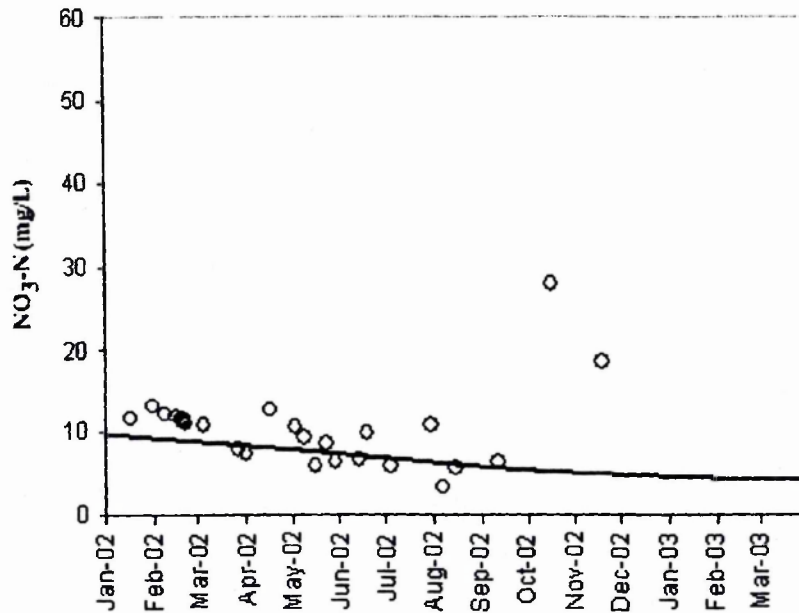
## 5.5 Model testing: simulation of denitrification at Chicheley

The parameters derived by calibrating INCA for the Bedford Ouse river between 2001 and 2003 were then used to put together a new set of parameters for the period 2002–2003. The parameter set derived is presented in Appendix XII. It was used with a different version of INCA, INCA-N Riparian, limited to one reach but including a riparian cell; this is an advance of the current version of INCA-N (Wade et al., 2002). The output flow and N flux is summed from the six land uses and delivered as input to the riparian cell, after allowing for any by-pass flow (which is set as a fixed fraction for the duration of the simulation).

The riparian cell has the same structure as the soil-component of the land-phase of the INCA-N model except denitrification is dependent on water-filled pore space (WFPS), rather than soil moisture deficit; since WFPS is more closely related to denitrification (Section 4.1) and is more commonly measured in the field. The residence times of flow and nitrogen for the land-use classes are user-input constants, and nitrogen is processed in the same manner as in the soil component of INCA-N. The output flow and nitrogen from the riparian zone cell are delivered to the stream, together with the by-pass component.  $\text{NO}_3\text{-N}$  dynamics and spatial variations in denitrification rates in the riparian cell were tested by comparing simulated stream  $\text{NO}_3\text{-N}$  concentrations and rates for 2002 to 2003, with corresponding observed values.

### NITRATE DYNAMICS

Observed and simulated  $\text{NO}_3\text{-N}$  concentrations for the stream are shown in Fig 5.6 for the Chicheley East denitrification site (Section 4.1).  $\text{NO}_3\text{-N}$  dynamics are generally represented adequately by model simulations.



**Figure 5.6:** INCA-N riparian simulation results for nitrate: observed and simulated NO<sub>3</sub>-N concentrations at Reach 1-CHEast for January 2002 to March 2003. Observed stream nitrate concentrations are shown as open circles; simulated stream nitrate concentrations are shown as a solid black line.

There was no clear seasonal pattern in the NO<sub>3</sub>-N concentrations at the Chicheley East denitrification site. Both observed and simulated NO<sub>3</sub>-N concentrations decreased slightly and showed little variation (Fig 5.6) during the study period.

#### POSITION-DEPENDENT DENITRIFICATION RATES IN THE RIPARIAN CELL

Denitrification rates in the riparian cell are simulated for the upper, intermediate, and lower (near-stream) positions of a slope. Three different moisture time series files for these positions were created using soil moisture measurements from the Chicheley East denitrification plot (Section 4.1). Soil moisture was measured on a weekly to fortnightly basis and interpolated in-between. An additional measured input value was soil bulk density, which is used together with soil moisture to calculate WFPS. The initial soil solution NO<sub>3</sub><sup>-</sup> concentration was first calibrated and then compared to measured values. Calibrated values are presented in Table 5.5. They are within the range of observed values (near-zero to about 60 mg N/L). Soil solution NO<sub>3</sub><sup>-</sup>

concentration measurements were taken from ceramic cups inserted 35 cm deep in the soil, and it is therefore difficult to compare these with the soil water  $\text{NO}_3^-$  concentrations in INCA since the model takes into account a 1-m-deep mixed soil.

The total annual denitrification rate measured at the three slope positions (Section 4.1) was used to compare to the output of the model. In Section 4.1, an annual denitrification rate of 5.0, 4.8 and 71.7  $\text{kg N ha}^{-1}$  was estimated at the intermediate, upper and lower levels respectively. The results also showed that the annual denitrification rate at the lower level of the slope was 15 times greater than at the other two levels because of a single high denitrification rate measured at that site at a high water-filled pore space of 70%, but that if the value was ignored the rate was still 4 to 5 times greater. All parameters and datafiles used in the model are given in Appendix XII.

**Table 5.5:** Moisture time series files (.mts), measured soil bulk density ( $\text{g/cm}^3$ ) and calibrated initial soil solution  $\text{NO}_3^-$  concentration (mg N/L) - for the lower, intermediate and upper levels of the slope. 'Approach 1' - same initial  $\text{NO}_3^-$  concentration for all slope positions; 'Approach 2' - higher initial  $\text{NO}_3^-$  concentration for the lower slope position, reflecting periodic flooding of high-  $\text{NO}_3^-$  stream water.

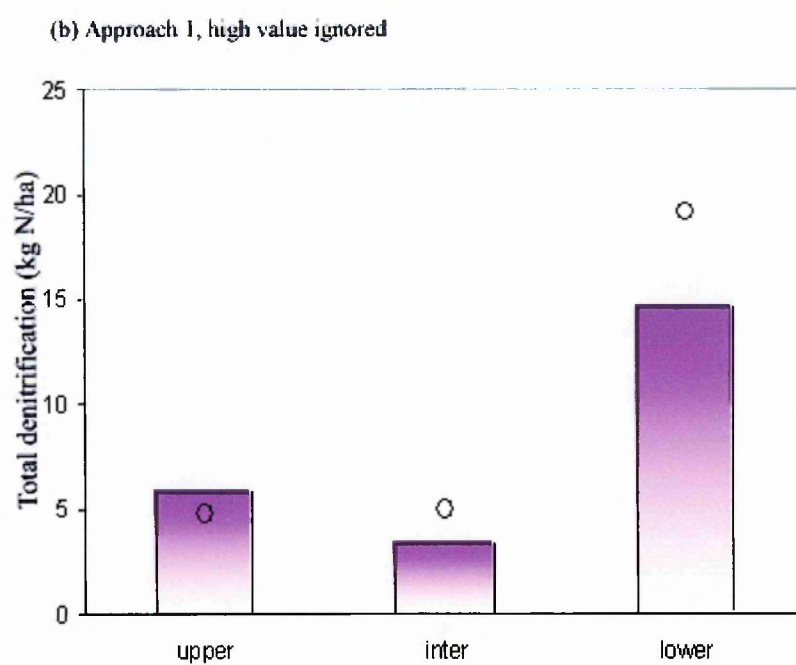
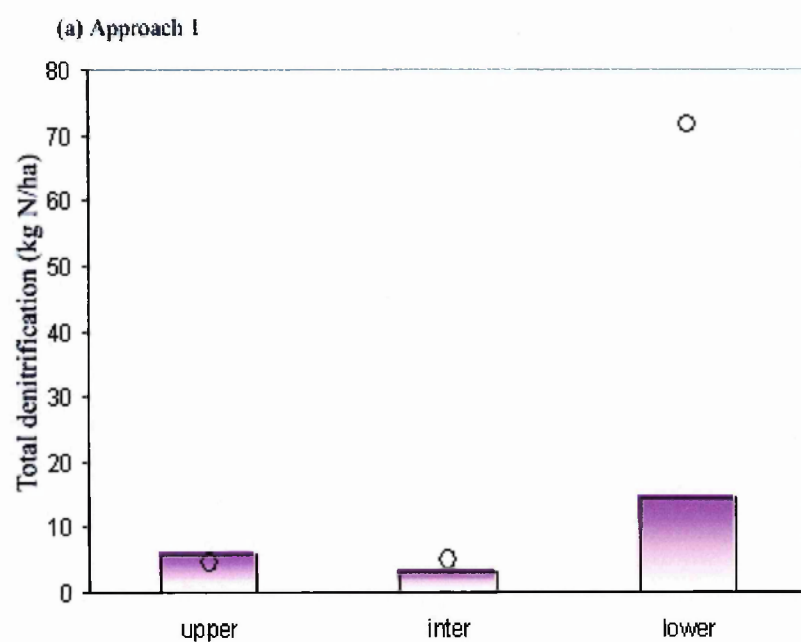
	Approach 1			Approach 2		
	Moisture Time Series (.mts)	Soil Bulk Density ( $\text{g/cm}^3$ )	Initial $\text{NO}_3^-$ concentration (mg N/L)	Moisture Time Series (.mts)	Soil Bulk Density ( $\text{g/cm}^3$ )	Initial $\text{NO}_3^-$ concentration (mg N/L)
Lower	lower	1.074	0.05	lower	1.074	15
Inter	inter	0.988	0.05	inter	0.988	0.05
Upper	upper	1.006	0.05	upper	1.006	0.05

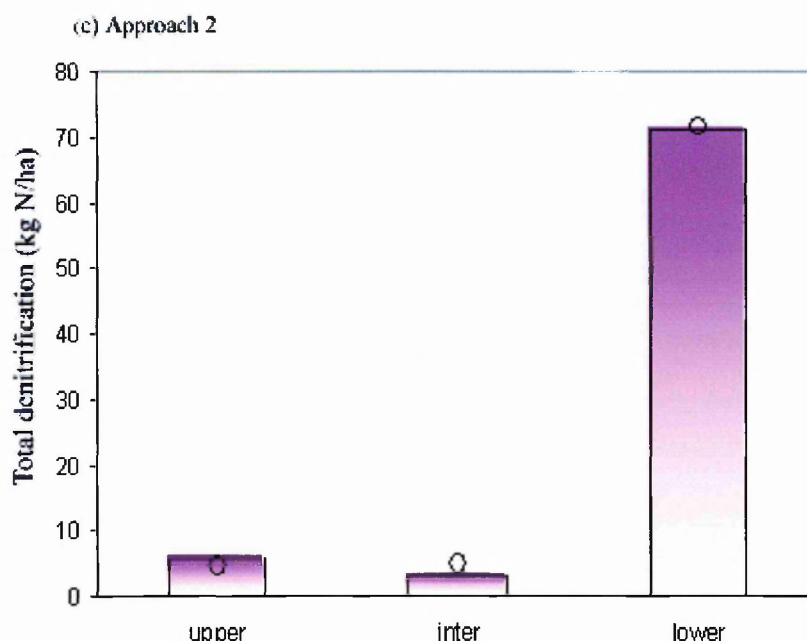
Total denitrification was well simulated by INCA-N Riparian at the two levels farthest from the stream (Figure 5.7a). For the lower-slope position, the model predicted a total denitrification rate of 14.63  $\text{kg N ha}^{-1}$ , 3 to 4 times greater than that simulated for the upper and intermediate levels. This was the result of a change of the soil bulk density

according to the level of the slope. In INCA-N Riparian changing the bulk density results in a change in water-filled pore space (%), which directly influences the denitrification rate. This is in good agreement with the annual flux estimated if the high value is ignored (Figure 5.7b). However, as was emphasized in Section 4.1.3, the high value corresponds to the denitrification rates expected at water-filled pore space of about 70%.

The high denitrification rate was measured after flooding had occurred at the lower level of the site. It is possible that the nitrate being denitrified is largely river water nitrate that has saturated the soil. The results from this first simulation show that the model needs a high  $\text{NO}_3^-$  concentration to generate rapid denitrification, and so the river is likely to be an important source. Generally, lower soil  $\text{NO}_3^-$  contents were observed at the lower slope nearest to the stream than at the two other locations of the slope at Chicheley East (denitrification plot). It is therefore likely that much of the nitrate denitrified at the lower level came from the river during flood events. The easiest way to simulate occasional flood-pulses of high-  $\text{NO}_3^-$  river water is to change the initial  $\text{NO}_3^-$  concentration at the lower level to a value that approaches streamwater  $\text{NO}_3^-$ , which can be as high as 30 mg N/L, but generally fluctuates around 10 mg N/L (Fig. 5.6). A value of 15 mg N/L provided the best fit in the model to the observed denitrification rate.

The total denitrification was well simulated at all three levels of the slope using this approach (Figure 5.7c).





**Figure 5.7:** INCA-N riparian simulation results for the total denitrification rates in the riparian cell at the upper, intermediate and lower levels of the slope for (a) Approach 1: different moisture time series and corresponding soil bulk densities but same initial  $\text{NO}_3^-$  concentration, (b) Approach 1: different moisture time series and corresponding soil bulk densities but same initial  $\text{NO}_3^-$  concentration, high observed denitrification value ignored and (c) Approach 2: different moisture time series and corresponding soil bulk densities with a different initial  $\text{NO}_3^-$  concentration at the lower level of the slope. Observed total denitrification rates are shown as open circles; simulated total denitrification rates are shown as bars.

## 5.6 Conclusion

INCA has proved to be a valuable tool for simulating catchment behaviour and was successfully calibrated to the Bedford Ouse catchment. A new version of the model, INCA-N Riparian, was tested for its ability to simulate total denitrification rate in a riparian cell. The first simulation was done with a set of parameters obtained from a version of INCA calibrated for the Bedford Ouse catchment. The results from this simulation did not fit the observations; therefore the soil initial  $\text{NO}_3^-$  concentration was modified based on the field observations. This version fitted the data well, showing that INCA can simulate background denitrification rates, but that it fails to simulate pulses of denitrification brought on by stochastic events such as floods or heavy rainfall. For the model to work well, nitrate concentrations at the lower slope position in the riparian



zone need to be larger than those measured with lysimeters at the site (mostly during non-flood events); this concentration is similar to the average river water concentration. This further supports the hypothesis that most of the denitrification at the lower level is of  $\text{NO}_3^-$  in river water. To improve INCA for riparian ecosystems, an exponential relationship between denitrification and WFPS should be incorporated in the model, as well as a capability to empirically simulate stochastic events such as heavy rainfalls. Daily measurements of soil water and river  $\text{NO}_3^-$  concentrations, as well as soil moisture would also be highly useful to better calibrate the model to simulate denitrification, since this process can vary greatly over short time intervals.

## **Chapter Six**

### **General discussion**

#### **6.1 Introduction**

The aims of the study were to (i) establish which of the factors (i.e. soil temperature, soil moisture, soil nitrate- and ammonium-N, soil organic carbon, rainfall) known to control the mitigation of nitrogen pollution to a river system by release of gaseous N, especially  $\text{N}_2\text{O}$ , has the major effect, (ii) determine how significant are riparian zones to  $\text{N}_2\text{O}$  release from agricultural land and how the nature of the riparian zone influences such release, (iii) assess denitrification in riparian ecosystems and determine which conditions favour the proportion of total nitrogen released by denitrification to shift from  $\text{N}_2\text{O}$  to  $\text{N}_2$  and (iv) empirically relate the  $\text{N}_2\text{O}$  fluxes and the denitrification rates to the main environmental controls and use the relationship found in INCA to estimate denitrification in riparian ecosystems.

Chapters 2-5 detail a variety of approaches that were used in order to examine the questions outlined in Chapter 1. Investigations were made under both natural

(Chapter 3) and laboratory controlled (Chapter 4) conditions. Finally, the implications of the findings were used to simulate denitrification in riparian ecosystems using the INCA model (Chapter 5).

In this chapter, the work is discussed and summarised and recommendations for future investigations are made.

## **6.2 Spatial and temporal variability of *in-situ* N<sub>2</sub>O fluxes**

*In-situ* N<sub>2</sub>O fluxes were measured at two experimental sites (Chapter 3) on a weekly to fortnightly basis using the closed chamber method and the results showed that nitrous oxide fluxes varied greatly both spatially and temporally, which was also observed in other temperate climate riparian ecosystems (Hanson *et al.*, 1994; Groffman and Tiedje, 1989). Generally, at Chicheley, fluxes were higher in spring/summer than in autumn/winter, since the spring/summer during the study period was quite wet. Differences in fluxes were observed between the two experimental sites. Fluxes at Chicheley East, sloping gently from the field to the stream, were generally higher than at Chicheley North, with a steep drop from the field.

At both locations, the highest rates of N<sub>2</sub>O emission were observed at the down slope positions. This is probably because the water table fluctuated closest to the surface at the down slope locations, especially at CH East which was sometimes flooded.

This large spatial and temporal variability is apparent in all measurements of nitrous oxide fluxes world-wide and in different types of ecosystems (Smith *et al.*, 1998; Dobbie *et al.*, 1999; Butterbach-Bahl *et al.*, 1998; Groffman *et al.*, 2000). The high variability of the fluxes was reported for nitrous oxide emissions from grassland and

arable soils in the UK (Dobbie *et al.*, 1999), from forest soils and agricultural soils in Germany (Butterbach-Bahl *et al.*, 1998; Mogge *et al.*, 1999) and from riparian zones in the USA (Groffman *et al.*, 2000).

## **6.3 Variables controlling rates of denitrification and N<sub>2</sub>O emissions**

Chapters 3 and 4 present and discuss the results from the experimental studies of field denitrification and N<sub>2</sub>O emissions in relation to the environmental factors thought to control both N<sub>2</sub>O releases.

### **6.3.1 Soil moisture**

The data obtained from N<sub>2</sub>O fluxes and denitrification measurements in the field along with soil water content (as volumetric soil moisture and water filled pore space) showed that soil moisture is a strong predictor for both N<sub>2</sub>O emissions and denitrification (Table 4.1), as long as NO<sub>3</sub><sup>-</sup> and temperature were above threshold levels. A clear non-linear pattern was observed between N<sub>2</sub>O fluxes and soil moisture (% vol.) or WFPS (%). N<sub>2</sub>O fluxes were very small until a threshold (24 % vol., 40 % WFPS) was reached, after which fluxes increased to a maximum at ca 35 % vol. or 65 % WFPS; then decreased with increasing soil moisture. In field experiments, Smith *et al.* (1998) also observed an increase in N<sub>2</sub>O emissions once a level of 50 % WFPS was reached and until > 90 %. Their results also showed a tendency for emissions to decline to much lower values at the highest water contents, attributable to reduction of N<sub>2</sub>O to N<sub>2</sub>, as reported by Focht (1978).

Field denitrification (Chapter 4) was found to be exponentially correlated to soil WFPS and soil volumetric moisture. However, the moisture threshold for denitrification was higher than for  $N_2O$  emissions and corresponded to 40 - 45 % vol. and 65 – 70 % WFPS (Figs 4.1a, b). When comparing these results with results from other studies, similar exponential trends were determined, with variable thresholds. These studies were undertaken in riparian ecosystems, investigated denitrification and its controls and differed in terms of experimental conditions (e.g. field/lab, temperature) and soil characteristics. These observations make a strong basis for the hypothesis that soil moisture is the main control on denitrification. A step-wise regression was also carried out on  $\ln$ -transformed variables and showed that WFPS was nearly always the best predictor for denitrification in Chicheley riparian ecosystem.

### *6.3.2 Soil nitrate*

The experimental plots where the study was carried out were riparian ecosystems situated on an active farm, draining agricultural lands subjected to fertiliser applications (Chapter 2). At these sites,  $NO_3^-$  in soil was always plentiful and had no limiting effect on the  $N_2O$  fluxes. However, higher fluxes were observed at the site receiving more fertiliser, potentially reflecting the greater application rates there. The experimental plots also differed morphologically, by the nature of their slope. It is possible that the difference observed in terms of the pattern of nitrate concentrations in soils along the slope between Chicheley East and Chicheley North reflects the difference in morphology.

In the study of field denitrification, soil nitrate was a secondary predictor of individual denitrification rates, after accounting for soil WFPS. However, both in this study and in the studies used for comparison, the fertilised sites showed denitrification rates

about 20 times greater than in unfertilised sites which lead to the conclusion that absolute rates of denitrification were related to soil  $\text{NO}_3^-$  concentrations.

### 6.3.3 Soil temperature

As part of the study presented in this thesis, soil temperature was one of the factors monitored in relation to  $\text{N}_2\text{O}$  fluxes and denitrification. As with soil moisture, no linear relationship was found between  $\text{N}_2\text{O}$  fluxes and soil temperature. However, the same type of non-linear pattern was observed. Fluxes were found to be significantly higher when temperatures exceeded  $8^\circ\text{C}$  ( $p < 0.001$ ) at both field sites. After this threshold was reached higher fluxes were observed on some occasions. Clearly, thresholds in soil moisture and soil temperature must be exceeded for  $\text{N}_2\text{O}$  fluxes to occur. However, even when these thresholds are exceeded there are still occasions when no fluxes occur. An explanation for this requires further research.

In contrast to  $\text{N}_2\text{O}$  fluxes, soil temperature was not found to be significantly correlated with denitrification rates. The step-wise regression (Chapter 4) rejected soil temperature as a possible predictor of denitrification at the experimental site. In a study of the regulators of denitrification in an organic riparian soil in New Zealand, Schipper *et al.* (1993) only found a weak ( $p < 0.12$ ) correlation between temperature and denitrification rates. Similar weak correlations between temperature and denitrification have been found by others (Davidson and Swank, 1986; Myrold, 1988; Parsons *et al.*, 1991).

Ambus (1993) reported the results from a study of the control of denitrification enzyme activity in a streamside soil in Copenhagen and his data showed that the temperature effect was more important than the effects caused by nitrate and carbon additions, but only when a change from  $0$  to  $15^\circ\text{C}$  was considered. The soil temperatures measured at the Chicheley experimental sites were very rarely below

the 8°C threshold and the overall temperature range was less than that considered to have an effect on denitrification by Ambus (1993). The differentiation between the direct effect of temperature changes on denitrification and indirect effects caused by the impact of temperature changes on for example respiration, nitrification and O<sub>2</sub> solubility, all of which are important factors in the regulation of denitrification, might often be impossible under *in situ* conditions. Therefore, the relative importance of temperature might change depending on the conditions.

#### 6.3.4 Dissolved Organic Carbon availability

Dissolved organic carbon (DOC) in the soil solution was measured alongside N<sub>2</sub>O fluxes. When the relationship between DOC and N<sub>2</sub>O fluxes was investigated, a similar trend to that observed for soil moisture and soil temperature was found. The data suggested a threshold of approximately 20 mg C L<sup>-1</sup> below which low fluxes occurred. Once this threshold was reached fluxes increased on some occasions, as with the soil temperature. However, low fluxes were still measured even above the DOC threshold, and the difference between N<sub>2</sub>O fluxes below and above this threshold was not significant at the 5 % probability level (Mann-Whitney Test). DOC was also included in a step-wise regression analysis and was not found to be a predictor of N<sub>2</sub>O emissions at Chicheley experimental sites. Mogge *et al.* (1999) studied the influence of organic fertilisers and land use on nitrous oxide emissions and denitrification N-losses from agricultural soils in the Bornhöved Lake region. Their results showed that temporal changes in the content of water-soluble organic-C compounds did not predict gaseous N-losses from the soils.

The results from the on-site denitrification study were examined in relation to DOC and showed that DOC was a significant predictor for denitrification only when denitrification was averaged on a position-in-the-slope basis. Conceptually, organic-

C, O<sub>2</sub> and NO<sub>3</sub><sup>-</sup> are the primary proximate regulators which dictate whether denitrification will occur (Tiedje, 1988). The rate of denitrification will be related to these regulators, to the number of active denitrifying bacteria present in the soil, and the soil temperature (Davidson *et al.*, 1990). In the study presented in this thesis, on-site denitrification was highly correlated to water filled pore space, soil nitrate and temperature.

#### 6.3.5 Stochastic factors: rainfall and flowpath

As part of the study of N<sub>2</sub>O emissions at the Chicheley experimental sites the effect of major rainfall events on the fluxes was observed. At both sites, large pulses of N<sub>2</sub>O occurred within two days following rainfall events  $\geq 10$  mm (Fig 3.2). This effect was only important if controls such as soil moisture, soil temperature and soil nitrate were above the thresholds identified in Chapter 3. However, the proportion of N<sub>2</sub>O emitted after major rainfall events accounted for most of the total annual N<sub>2</sub>O at both sites. This effect has not yet been widely studied, however, the observation that N<sub>2</sub>O fluxes respond to rainfall has already been made. Dick *et al.* (2001) looked at the effect of rainfall on NO and N<sub>2</sub>O emissions from Ugandan agroforest soils. They showed that a single 25 mm rain event could stimulate a large N<sub>2</sub>O pulse after a delay of one day. The same response has been observed following wetting of a dry soil (Davidson *et al.*, 1993, Scholes *et al.*, 1997). Experiments where rainfall is simulated are needed to further investigate this effect in a range of different ecosystems and estimate the contribution of such emissions to global N<sub>2</sub>O emissions, which might be underestimated at present.

Another stochastic factor is flowpath. At Chicheley North, the nitrate concentration in the soil solution was higher at the lower location of the slope than at the location farthest to the stream. This is the opposite of what was observed at Chicheley East



where  $\text{NO}_3^-$  is removed from percolating water as it moves down slope before it reaches the stream. There is a possibility that a source of nitrate between the upper and lower slope locations exists at that site, and that nitrate-rich groundwater from the field is by-passing the upper slope location.

## 6.4 Modelling denitrification

INCA has proved to be a valuable tool for simulating catchment behaviour. It appears to reproduce the broad patterns of hydrology and  $\text{NO}_3^-$  leaching and changes in land use, hydrology and deposition generate reasonable and acceptable results. INCA was successfully calibrated to the Bedford Ouse and the calibration was used to derive a set of parameters then used with an advance of the current version of INCA-N (Wade et al., 2002). In the riparian version of INCA, INCA-N Riparian, a cell representing the riparian zone was added to the current INCA-N model (Chapter 5, section 5).  $\text{NO}_3\text{-N}$  dynamics and spatial variations in denitrification rates in the riparian cell were tested by comparing simulated  $\text{NO}_3\text{-N}$  concentrations and rates for the study period, with corresponding observed  $\text{NO}_3\text{-N}$  concentrations and rates for the study period. The INCA-N Riparian model was first tested using the daily moisture time series corresponding to each level of the slope, changing the bulk density of the soil accordingly and keeping all other parameters. The simulation did not fit the observations and the total denitrification at the lower slope was underestimated. The field results showed that the annual denitrification rate at the lower level of the slope was 15 times greater than at the other two levels because of a single high denitrification rate measured at that site at a high water-filled pore space of 70%. Ignoring this value resulted in a denitrification rate 4 times higher at the lower level. This was well simulated by the model. However, the soil  $\text{NO}_3^-$  initial condition

was changed to 15 mg N/L at the lower location of the slope in order to generate the high denitrification rate at the near-stream location. In the study, the one high denitrification measurement was made after flooding occurred. INCA-N Riparian needed a high nitrate concentration to generate rapid denitrification suggesting that most of the denitrification was of nitrate in river water saturating the soil.

The work discussed so far provides strong evidence that soil moisture as water-filled pore space (% WFPS) is the environmental control that has the major effect on the release of gaseous N, especially  $N_2O$ , from riparian ecosystems and that it is the best predictor of both *in-situ*  $N_2O$  emissions and denitrification. This is further supported by a number of other studies from the literature compiled for comparison.  $N_2O$  emissions were mainly controlled by water-filled pore space and temperature and nitrate played secondary roles. The study emphasizes the importance of stochastic factors such as rainfall events or flooding which have not yet been widely investigated but were found to account for as much as 70 – 90 % of the total  $N_2O$  emitted at the Chicheley experimental sites. In addition, the results presented in this thesis suggest that denitrification rates can be simply modelled by using a general exponential relationship between denitrification rates and water-filled pore space multiplied by a constant value depending upon the N status of the site. Such relationship should be used in INCA to simulate denitrification in riparian ecosystems. The study presented here demonstrates that riparian ecosystems should be taken into account when investigating global budgets of  $N_2O$  since they are an important component of the  $N_2O$  release from agricultural land. Ignoring such sources could result in great underestimation of  $N_2O$  emission estimates when using current tools such as emission factors. This study was conducted on two different riparian ecosystems and the results suggest that differences in morphology or N status of the site have an impact on the efficiency of the site to mitigate  $NO_3^-$  pollution to river water or release of  $N_2O$  to the atmosphere. It is an important aspect that should be

carefully investigated. At the experimental site, the end product of denitrification was mainly  $N_2$  when the water-filled pore space was above 60%. This is an important finding and gives an insight as to what condition favours the proportion of total nitrogen released by denitrification to shift from  $N_2O$  to  $N_2$ .

## 6.5 Recommendations for future work

- Similar long-term *in-situ* measurements of  $N_2O$  fluxes along with the environmental controls, as reported in this thesis, should be conducted in riparian ecosystems over larger areas such as whole catchments in order to investigate the possibility of scaling up these findings and, therefore, a scaling up within INCA.
- This study suggests that thresholds for the main predictors of  $N_2O$  fluxes and denitrification rates seem necessary for these fluxes and rates to occur but there is an important scatter, for example when high moisture contents are reached. This requires further investigation.
- Similar studies as that presented in this thesis (Chapters 3 and 4) in riparian ecosystems spanning different climatic regimes (e.g. the countries within the INCA frame) are needed to create a "European map" of riparian  $N_2O$  fluxes and denitrification to global  $N_2O$  production.
- *In-situ* denitrification plays an important role in the release of  $N_2O$  from riparian ecosystems and therefore requires further long-term intensive monitoring.

- There is a need for intensive measurements of both  $\text{N}_2\text{O}$  fluxes and denitrification on the short term scale in order to better capture the great temporal variations in emissions.
- The findings presented in this thesis (Chapter 3) suggest that rainfall events are a major control of  $\text{N}_2\text{O}$  fluxes. Experiments including manipulations to simulate rainfall are required in order to further investigate this interaction.
- The riparian module of INCA-N has limitations when trying to simulate total denitrification at the different levels of the slope in the riparian cell. It can not simulate stochastic events such as rainfall pulses, and can not deal with flow of river water into the bank. Further work is therefore needed towards including these in INCA-N Riparian and achieving a good prediction of the denitrification rates.
- Riparian buffers are currently included in government policies for controlling diffuse pollution, but the scientific understanding to make them efficient in terms of nitrate removal as well as limiting their contributions to the  $\text{N}_2\text{O}$  release is still lacking. This clearly deserves further investigation.
- The studies presented in this thesis (Chapters 3 and 4) were done in agricultural riparian ecosystems. Similar studies as that presented in this thesis in different types of riparian ecosystems, such as forested or herbaceous, would allow greater knowledge on how the vegetation cover of the riparian ecosystem influences  $\text{N}_2\text{O}$  emissions and denitrification.

- Other processes, such as nitrification and nitrifier denitrification (Chapter 1), may also contribute to the N<sub>2</sub>O emissions from riparian ecosystems. They should, therefore, be examined for their potential contribution over the short and long-term.

## 6.6 Summary and conclusions

- In the two-year field experiments, N<sub>2</sub>O fluxes were found to be higher at Chicheley East than at Chicheley North possibly due to a higher fertiliser application rate as well as the difference in morphology.
- Soil moisture (% vol. as well as WFPS) was found to be the main control of N<sub>2</sub>O emissions at both sites. At soil moistures below 24 % vol. small fluxes were observed and increased after this threshold was reached. On some occasions low fluxes were still recorded above the threshold if the other secondary controls were limiting.
- Soil temperature and DOC were secondary controls of field N<sub>2</sub>O emissions. Soil nitrate was plentiful and never limited N<sub>2</sub>O fluxes at both sites. The thresholds for soil temperature and DOC determined in this study were 8°C and 20 mg C L<sup>-1</sup>, respectively.
- In the 12-month acetylene inhibition experiment, field denitrification was exponentially correlated to the water-filled pore space (% WFPS) of the soil. This is in agreement with findings compiled from the literature. All studies considered showed similar threshold values of 60-80 % WFPS, but different

absolute rates of denitrification. The absolute rates were related to the soil nitrate concentrations. This result suggests that denitrification could be simply modelled by using a general exponential relationship between denitrification rates and water-filled pore space multiplied by a constant value depending upon the nitrogen status of the site.

- For soil volumetric water contents below 35%, gaseous N was emitted as 50:50 (%) /  $\text{N}_2\text{O}$ :  $\text{N}_2$  at all three levels of the slope above the stream surface. Above this moisture level, gaseous N was mainly emitted as  $\text{N}_2$ .
- Denitrifying Enzyme Activity (DEA) varied according to the season and was highest in summer 2002. It then decreased in November 2002 and either increased or remained stable in March 2003. The results indicated that even when field conditions are limiting denitrification, the soil denitrifier population and the potential for denitrification in the soil persist.
- DEA and denitrification did not correlate. This can be interpreted as a consequence of the fact that only a small percentage of denitrifying enzymes are active at any one time in a soil and that the occurrence of denitrifying bacteria in any given habitat is determined more by their ability to compete as heterotrophs rather than by their ability to denitrify. It is also possible that the field denitrification measurements presented here did not represent the overall rates, and that more intensive measurements are needed.
- $\text{N}_2\text{O}$  pulses following rainfall were found to contribute 70-90% of the total annual  $\text{N}_2\text{O}$  at both experimental sites. Further investigations are needed at

the time to account for this response and avoid underestimating global emissions.

- Another aim of this study was to evaluate the usefulness of emission factors as a tool for setting pollutant control legislation. Protocols have been developed by the Intergovernmental Panel on Climate Change (IPCC, 1997) that provides a methodology for calculating emissions using defined emission factors. At present, the IPCC assumes there are three principal sources for agricultural  $\text{N}_2\text{O}$  emissions which are: direct emissions from soil nitrogen (N); emissions from animal waste management systems and indirect emissions from N lost to the agricultural system (see Chapter 3). This study suggests that emission factors, although useful to estimate current rates and changes in  $\text{N}_2\text{O}$  emissions, could introduce great uncertainties and lead to an important underestimation of such emissions if they are not taking into account  $\text{N}_2\text{O}$  sources like riparian ecosystems.
- INCA was successfully calibrated to the Bedford Ouse and a newly implemented version of the model, INCA-N Riparian, including a riparian cell at the catchment-river interface was used to simulate total denitrification rates. The results were compared to the experimental measurements presented in this thesis. The model showed that background denitrification rates could be simulated. However, it needed a higher input value for the initial soil nitrate concentration in order to simulate the much higher denitrification rate observed at the position of the slope nearest to the stream, suggesting that denitrification at that location was mainly of river nitrate saturating the soil.

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## Appendix I

Nitrous oxide emission rates in Europe, ecosystem types and soil characteristics

Country	Ecosystem type Measurement period	Soil type	Annual N <sub>2</sub> O rate (kgN <sub>2</sub> O- N/ha/yr)	monitored parameters	pH	soil temp	moisture	Corg	N inputs	rainfall	literature reference
							%	Mg C/g soil	kgN/ha	mm yr <sup>-1</sup>	
Denmark	Riparian grassland 3 yrs (1990-1992)	well to poorly drained organic soils	0.66	moisture, inorganic N, SOM pH	8	8.3	57 (vol.)	303	-	800	Ambus and Christensen, 1995
	Spruce forest 3 yrs (1990-1992)	well drained loamy sand	0.77	moisture, inorganic N, SOM pH	4	8.45	60 (vol.)	369	-	800	Ambus and Christensen, 1995
	Beech forest 3 yrs (1990-1992)	well drained sandy loam	0.8	moisture, inorganic N, SOM pH	5.6	7.9	54 (vol.)	36.3	-	800	Ambus and Christensen, 1995
	Coastal grassland 3 yrs (1990-1992)	well to poorly drained sandy soil	1.24	moisture, inorganic N, SOM pH	7	7.25	86 (vol.)	120	-	800	Ambus and Christensen, 1995
	Upland arable fertilised 3 yrs (1990-1992)	somewhat to poorly drained sandy loam	3.61	soil moisture, inorganic N, pH SOM	7.6	9.75	69 (vol.)	164	180	800	Ambus and Christensen, 1995
	Drained arable fertilised 3 yrs (1990-1992)	poorly drained loam	4.67	soil moisture, inorganic N, pH SOM	7.7	10.95	77 (vol.)	164	110	800	Ambus and Christensen, 1995
Finland	Coniferous forest (1991-1992)	mineral soil, low N deposition	0.122	pH, soil temperature	<6	8.5	-	-	30	600	Martikainen <i>et al.</i> , 1994
	Peatlands (1991-1992)	peat soils organic	1.35	total N, pH, water table, P, K, Ca contents	4.4	8.5	-	-	-	-	Regina <i>et al.</i> , 1996
	Spruce forest clearcutting, drainage area (1993-1994)	peat soils underlain by sandy till/clay organic	1.47	total N, inorganic N, soil temperature	acid	7	-	-	6.25	607	Nieminen, 1998

Continued									
Finland	Forested peatlands (1991-1992)	peat soil organic	1.7	total N, pH, water table, P, K, Ca contents	4.4	8.5	-	-	Regina <i>et al.</i> , 1996
	Grass (cultivation), high N Content (1991-1992)	peat soil organic	14.54	total N, pH, water table, P, K, Ca contents	5.3	8.5	-	-	Regina <i>et al.</i> , 1996
	Agricultural, low N Deposition (1991-1992)	mineral soils organic	0.929 23.23	pH, soil temperature	acid	8.5	-	-	Martikainen <i>et al.</i> , 1994
France	Agricultural (seeded, unfertilised) 12 months (1994-1995)	3 soils*	0.14	moisture, soil temperature and soil nitrate	7.3	10.5	75 (WFPS)	18.7	Henault <i>et al.</i> , 1998
	Agricultural (bare soil, herbicide) 12 months (1994-1995)	3 soils*	3.513	moisture, soil temperature and soil nitrate	7.3	10.5	75 (WFPS)	18.7	Henault <i>et al.</i> , 1998
	Agricultural (rapeseed, suboptimally fertilised) 12 months (1994-1995)	3 soils*	15.9	moisture, soil temperature and soil nitrate	7.3	10.5	75 (WFPS)	18.7	153 Henault <i>et al.</i> , 1998
	Agricultural (rapeseed, overfertilised) 12 months (1994-1995)	3 soils*	18.52	moisture, soil temperature and soil nitrate	7.3	10.5	75 (WFPS)	18.7	257 Henault <i>et al.</i> , 1998
Germany	Beech forest 12 months (1993)	loamy sand sedimentary origine	0.4	moisture, soil temperature, nitrate, water soluble organic C	4	9	50 (wt/wt)	34	23.8 697 Mogge <i>et al.</i> , 1998

\*: typic rendzic leptosol, gleyic luvisol, eutric leptosol  
 Temperatures are annual means except for the following studies: Henault *et al.*, 1998 (3 months), Martikainen *et al.*, 1994 (9 months), Regina *et al.*, 1996 (9 months)

## Continued

Germany	Grassland, N input 12 months (1993)	sedimentary origine, loamy sand	1.5	soil moisture, nitrate, pH soil temperature, water soluble organic C	6.4	11.5	30 (wt/wt)	16.5	78	697	Mogge et al., 1999
	Beech forest 3 yrs (1994-1996)	very acid hapludalf	3.81	soil ammonium and nitrate, soil moisture and temperature	4	6.65	33.5 (WFPS)	-	20	800	Papen and Butterbach-Bahl, 1999
	Black Alder forest 12 months (1993)	forest soil on peat	4.9	pH, moisture, soil temperature, nitrate, water soluble organic C	4	9	70 (wt/wt)	422	69	829	Mogge et al., 1998
	Agricultural (crop rotation field), farmyard manure fertilised 12 months (1993)	loamy sand	5.3	soil water, nitrate, temperature water soluble organic C	6.4	11.5	50 (wt/wt)	12.8	79	697	Mogge et al., 1999
	Arable, fertilised (FYM, CaNH <sub>4</sub> NO <sub>3</sub> ) 12 months (1993)	sandy and clay soils	13.08	soil temperature, nitrate, moisture	6.1	-	-	16.9	124	833	Flessa et al., 1995
UK	Sitka Spruce forest 3 yrs (1992-1994)	brown forest soil	0.35	pH, moisture, soil nitrate and ammonium, soil temperature	3.9	10	29 (wt/wt)	-	58	800	Skiba et al., 1996
	Long term grass sward Plots with dung and urine 15 months (1994-1995)	poorly drained silty clay loam	0.359	moisture, pH, soil temperature, soil available NO <sub>3</sub> -N, NH <sub>4</sub> -N	5.5	11	-	-	-	1050	Yamulki et al., 1998
	Pine forest Sitka Spruce, N inputs 3 yrs (1992-1994)	brown forest soil	0.36	pH, moisture, soil nitrate and ammonium, soil temperature	3.5	11	34 (wt/wt)	-	10	800	Skiba et al., 1996
	Birch forest 3 yrs (1992-1994)	brown forest soil	0.6		4.9	10	26 (wt/wt)	-	10	800	Skiba et al., 1996
	Mowed grass 3 yrs (1992-1994)	brown forest soil	0.88	soil moisture, pH, available ammonium and nitrate	4.8	11	30 (wt/wt)	-	10	800	Skiba et al., 1996



## Appendix II

### N<sub>2</sub>O analysis – gas chromatography

Gas chromatography was used throughout the study to measure concentrations of N<sub>2</sub>O. This method relies on the ability of a porous column (stationary phase) to partition different compounds that may be introduced to a carrier gas (mobile phase) which is passing through the column. Individual components within the introduced sample are retarded for different lengths of time depending on the extent of interaction between the component and the column with components emerging in order of increasing interaction with the column.

When N<sub>2</sub>O has emerged from the column it is detected on an electron capture detector (ECD). The radioactive element inside the ECD detector emits electrons (beta particles) which collide with and ionise some of the carrier gas. This reaction forms a stable cloud of free electrons in the ECD detector cell. The ECD electronics work to maintain a constant current equal to the standing current through the electron cloud by applying a periodic pulse to the anode and cathode. The standing current value is selected by the operator; the standing current value sets the pulse rate through the ECD cell. If the current drops below the set standing current value, the number of pulses per second increases to maintain the standing current. When electronegative compounds enter the ECD cell from the column, they immediately combine with some of the free electrons, temporarily reducing the number remaining in the electron cloud. When the electron population is decreased, the pulse rate is increased to maintain a constant current equal to the standing current. The pulse rate is converted to an analog output, which is acquired by the data system. Unlike other detectors which measure an increase in signal response, the ECD detector electronics measure the pulse rate needed to maintain the standing current.



## Appendix III

### Denitrifying enzyme activity (DEA) – experimental protocol

#### *Assay*

1. Sample fresh soil, ~200g, bulk sample for each level of the slope, at 0-10 cm depth.
2. In lab: sieve soil samples (remove root material etc...), weigh triplicate 20g of soil for each sample into 125ml-Erlenmeyer flasks. Add 20ml media (see media preparation).
3. Prepare 2 controls: flasks + media.
4. Seal flasks and make soils anaerobic: evacuation and flushing (3 cycles of 1 minute or whatever time is suitable depending on the capacity of the pump used) with Oxygen-free gas ( $N_2$ ).
5. Bring flasks to atmospheric pressure (at the final flushing with oxygen-free gas, use a sampling bag at atmospheric pressure and leave to equilibrate).
6. Add acetylene to 10% of the volume of the headspace of the flask.
7. Place flasks on shaker (125 rpm) at constant temperature (measure temperature at the same time).
8. Take samples at 0, 30, 60, 90 and 120 minutes (10 ml into Exetainers evacuated prior to experiment) after 10 ml of  $N_2$  gas have been added to the flask.
9. Analyse on GC.

#### *Media preparation*

Preparation of one litre of solution containing 100 mg  $KNO_3$ -N/kg dry soil, 500 mg glucose-C/kg dry soil and 100 mg chloramphenicol/kg dry soil.

$KNO_3$  : 101.107 g/mol      N: 14.01 g/mol

Glucose ( $C_6H_{12}O_6$ ): 180.16 g/mol      C: 12.01 g/mol

To obtain a 1L-solution as above, dissolve:

100 mg of chloramphenicol, 0.722 g of KNO<sub>3</sub> and 1.25 g of glucose into 1 litre of ultra pure water.

#### *Purification of acetylene*

Acetylene industrial grade was passed through one Analar H<sub>2</sub>SO<sub>4</sub> conc. trap (~100 ml) and a trap of ultra pure water (~100 ml). Purified acetylene was then collected in gas-tight bags.

#### *Calculations*

$$\text{Denitrification Rate} = [(M_{90} \times H) - (M_{30} \times H)] / (D \times T)$$

where DR is in µg/kg soil/h, H is the flask headspace volume (mL), D is the soil dry weight (g), T is the incubation time (min), M<sub>90</sub> and M<sub>30</sub> are the N<sub>2</sub>O concentrations at 90 and 30 minutes, respectively, to account for the amount of N<sub>2</sub>O dissolved in the liquid phase.

$$M_g = C_g \times (V_g + V_l \times b)$$

where M is the total amount of N<sub>2</sub>O in water + gas phase, b is the Bunsen coefficient (Table 2.1), V<sub>g</sub> is the volume of the gas phase, V<sub>l</sub> is the volume of the liquid phase and C<sub>g</sub> is the N<sub>2</sub>O concentration in the gas phase.

## **Appendix IV**

Definition of terms related to soil water content

	Definition	Mathematical expression
Gravimetric moisture	Instantaneous water content of soils on a weight basis (g water/g dry soil)	$\theta_g = [(g \text{ moist soil}) - (g \text{ dry soil})] / (g \text{ dry soil})$
Volumetric moisture	Determination of the volume of water by volume of soil (mL water/cm <sup>3</sup> soil)	$\theta_v = \theta_g \times \text{Bulk Density}$ with Bulk Density in g dry soil/cm <sup>3</sup> soil
Time Domain Reflectometry (TDR)	Measurement of the soil dielectric constant that is generally proportional to the water content. Gives the volumetric water content.	-----
Water-Filled Pore Space	Measurement of field water content. Gives the percentage of soil pores filled by water.	% WFPS = $[P_w \times (BD / S_t)] \times 100$ with $P_w = \theta_g \times 100$ , Bulk Density (BD) in g dry soil/cm <sup>3</sup> soil and Total porosity ( $S_t$ ) as %
Water Holding Capacity	Amount of water held by the soil after the water held by this soil at field capacity has drained away.	% WHC = $[g \text{ water retained by soil} \times 100] / (g \text{ oven dry soil})$

### *General principle*

Direct or indirect measures of soil water content (Appendix III) are needed in practically every type of soil study. In the field, knowledge of the water available to plant growth requires a direct measure of water content or a measure of some index of water content. It is also important since the moisture status of a soil will be decisive in terms of the occurrence of biogeochemical processes. In the laboratory, determining and reporting many physical and chemical properties of soil necessitates knowledge of water content. In soils work, water content traditionally has been expressed as the ratio of the mass of water present in a sample to the mass of the sample after it has been dried to constant weight, or as the volume of water present in a unit volume of the sample. In either case the amount of water in the sample is needed. To determine this, the water must be removed and measured, or the mass of the sample must be determined before and after removal of water.

Water content as usually used in soils work is either a dimensionless ratio of two masses or two volumes or is given as a mass per unit volume. When either of the dimensionless ratios is multiplied by 100, such values become percentages, and the basis (mass or volume) should be stated. Where no indication is given, the figure generally may be assumed to be on a mass basis because the determination usually involves getting mass-basis figures first and then converting them to volume-basis figures.

### *Gravimetric water content*

The procedure to be used varies with the circumstances of the measurement and the equipment. Where moderate precision (e.g., measurements having a precision of  $\pm 0.5\%$  water content) is desired the following procedure should be used. Samples should be duplicated as a minimum. Place sample of 1 to 100 g of soil in weighing bottles or metal cans with tight-fitting lids. Weigh the sample immediately, or store them

in such a way that evaporation is negligible. Place the sample in a drying oven with the lid off, and dry it to constant weight. Remove the sample from the oven, replace the cover, and place it in a desiccator until cool. Weigh it again, and also determine the tare weight of the sample container. Compute the water content by the following formula:

$$\theta_{dw} = (\text{weight of wet soil} - \text{weight of dry soil}) / \text{weight of dry soil}$$

Water filled pore space calculation

$$\% \text{ WFPS} = [P_w \times (BD/S_t)] \times 100$$

where

% WFPS = percent water-filled pore space

$P_w$  = water content ( $[\text{g water} / \text{g dry soil}] \times 100$ )

BD = bulk density ( $\text{g}/\text{cm}^3$ )

$S_t$  = total porosity (%)

$$\text{Total porosity (\%)} = [1 - (\text{bulk density} / \text{particle density})] \times 100$$

where

particle density = assumed to be  $2.65 \text{ g}/\text{cm}^3$ , for most mineral soils.

Volumetric water content calculation

$$\theta_v = \theta_g \times BD$$

where

$\theta_v$  = volumetric water content as  $\text{mL H}_2\text{O}/\text{cm}^3$  soil

$\theta_g$  = gravimetric water content as  $\text{g H}_2\text{O} / \text{g dry soil}$

BD = soil bulk density as  $\text{g dry soil}/\text{cm}^3$

If high precision is not required, one can assume clay soils have bulk densities around  $1.1 \text{ g}/\text{cm}^3$  and soils high in sand have bulk densities nearer  $1.7 \text{ g}/\text{cm}^3$ .

## **Appendix V**

### **Soil pH measurement**

The soil pH was measured after shaking one part of the soil by weight with 2.5 parts of distilled water by volume and leaving stand for 30 minutes and immersing the tips of a glass and a calomel reference electrode in the supernatant solution.

## Appendix VI

### Particle size analysis (PSA) – general principle

Particle size analysis (PSA) is a measurement of the size distribution of individual particles in a soil sample. The major features of PSA are dispersion of soil aggregates into discrete units by chemical, mechanical or ultrasonic means and the separation of particles according to size limits by sieving and sedimentation. Soil particles cover a size range varying from stones and rocks (exceeding 0.25 m in size) down to submicron clays ( $< 1 \mu\text{m}$ ). Various systems of size classification have been used to define arbitrary limits and ranges of soil particle size. Soil particles smaller than 2000  $\mu\text{m}$  are generally divided into three major size groups: sands, silts and clays.

### Particle size analysis (PSA) – experimental protocol

#### 1 – Soil preparation

- (i) Soils need to be air dried and sieved  $< 2\text{mm}$ .
- (ii) Analysis carried out on an oven-dried basis so soils (40g) need to be dried in the oven at  $103^{\circ}\text{C}$  overnight. Record weight: start weight.

#### 2 – Organic Matter and carbonate removal (most soils need it)

- (i) Add 30–40 mL water into 500 mL beaker where soil is then acidified (few drops of 1M HCl) to pH 3.5–4. Leave for 10–15 min.
- (ii) Add 10 mL of 100 vol (30%)  $\text{H}_2\text{O}_2$ , cover beaker with watch glass, leave for 40–60 min (until frothing ceases). Add another 10 mL unless still frothing and leave for 40–60 min.



- (iii) Heat up to 90°C, add another 10 mL of H<sub>2</sub>O<sub>2</sub> leave for 40-60 min. Cover with watch glass and allow to cool. Check solution is clear.
- (iv) Quantitatively transfer to pre-weighed centrifuge tubes (vol up to 100 mL)
- (v) Centrifuge at 2,000 rpm for 15 min.
- (vi) Remove clear supernatant liquid. If clay remains suspended, add a few drops of 0.5 M MgCl<sub>2</sub>, mix suspension without disturbing sedimented material, centrifuge tubes and decant the supernatant liquid.
- (vii) Add water to centrifuge tubes and centrifuge again. Decant and discard supernatant.
- (viii) Put tubes + sedimented material into oven at 103°C overnight. Record weight.

### 3 – Dispersion

- (i) Transfer dried treated soil to plastic bottles (500 mL, labelled) and add 100 mL of 5% HexaMetaPhosphate solution (50g of HMP powder in 1L, mix thoroughly using magnetic stirrer).
- (ii) Shake overnight.

### 4 – Sedimentation

- (i) Wash samples through 53 micron sieve using < 1L distilled water (make sure sieve is wet prior to sieving dispersed soil).
- (ii) Collect water plus sample that passes the sieve and place in a 1L sedimentation cylinder using a plastic funnel. Rinse and make volume up to 1L with distilled water.
- (iii) Place the captured particles (sand, any coarse particulate organic matter left) in pre-weighed aluminium pan and dry at 103°C overnight.
- (iv) Add 100 mL of 5% HexaMetaPhosphate solution to a sedimentation cylinder and add distilled water to 1L: this is your blank.

- (v) Let cylinders equilibrate thermally. Record temperature.
- (vi) Mix all cylinders thoroughly for about 30s (either using manual stirrer or by applying bung to the cylinder and turning it upside down a few times).  
If the surface of suspension is covered with foam add a drop of amyl alcohol.
- (vii) As soon as mixing is completed, lower hydrometer into suspension and take readings at 30s and again after 1min. Remove hydrometer, rinse and wipe. Reinsert hydrometer carefully about 10s before each reading and take readings at 90 and 1440 mins (1 hr 30 and 24 hrs). Read blank and record temperature each time.
- (viii) Transfer the dried sand to the nest of sieves arranged from top to bottom in the following order: 1000, 500, 250, 125 and 53 micron. Shake on a sieve shaker for 3mins. Weigh each of the sand fraction and the residual silt and clay that have passed through the 53micron sieve.

## 5 – Calculations

- (i) Clay fraction procedure

Take hydrometer readings at 1.5 and 24 h (record both R and  $R_L$  values). Determine the effective particle diameter X and summation percentage P for 1.5- and 24-h readings using the following equations:

$$P_{2\mu m} = m \ln (2/X_{24}) + P_{24} \quad (1)$$

where

$X_{24}$  = mean particle diameter in suspension at 24 h

$P_{24}$  = summation percentage at 24 h

$$m = (P_{1.5} - P_{24}) / \ln (X_{1.5}/X_{24}) = \text{slope of the summation percentage curve}$$

between X at 1.5 h and X at 24 h,

$X_{1.5}$  = mean particle diameter in suspension at 1.5 h, and

$P_{1.5}$  = summation percentage at 1.5 h.

$$X = \theta t^{1/2} \quad (2)$$

where  $\theta$  is the sedimentation parameter and is a function of the hydrometer settling depth, solution viscosity, and particle and solution density.

$$\theta = (18\eta h' / [g(\rho_s - \rho_l)])^{1/2} \quad (3)$$

where  $h'$  = hydrometer settling depth, cm,  $\eta$  = fluid viscosity,  $\rho_s$  = particle density,  $\rho_l$  = liquid density, and  $g$  = acceleration due to gravity.

$h' = -0.39R + 19.9$  for the BS hydrometer used.

$$P = [(R - R_L)/C_o] \times 100 \quad (4)$$

where  $R$  = uncorrected hydrometer reading in g/L,  $R_L$  = hydrometer reading of a blank solution in g/L and  $C_o$  = oven-dry weight of the soil sample (g)

#### (ii) Sand fraction calculation

Compute the 50- $\mu\text{m}$  summation percentage, using the same procedure as for  $P_{2\mu\text{m}}$ , but use the 30- and 60-s hydrometer readings rather than the 1.5- and 24-h readings, respectively, and subtract the computed  $P_{50\mu\text{m}}$  value from 100 to obtain the sand percentages. A standard sieve analysis should be run for comparison, using the 53- $\mu\text{m}$  screen.

$$\text{Sand percentage} = (\text{sand mass}/C_o) \times 100$$

where sand mass = dry mass of material captured on the 53- $\mu\text{m}$  sieve, and  $C_o$  = dry mass of the soil sample.

**(iii) Silt fraction calculation**

**Determine the percent silt by difference as**

$$\% \text{ silt} = 100 - (\% \text{ sand} + \% \text{ clay}).$$

## Appendix VII

### Dissolved ions

#### *Principle*

All chromatographic methods share the same basic principles and mode of operation. The sample to be analyzed (the analyte) is applied to some stationary fixed material (the adsorbent) and then a second material (the eluent) is passed through or over the stationary phase. The compounds contained in the analyte are then partitioned between the stationary adsorbent and the moving eluent. The success of the method depends on the fact that different compounds adhere to the adsorbent with different forces and are therefore moved through the adsorbent at different rates as the eluent flows over them. So, as the eluent flows through the column, the components of the analyte will move down the column at different speeds and therefore separate from one another. Components of the fastest moving substance (least tightly bound to the adsorbent) will be observed emerging from the column - usually in a narrow band initially but with later compounds being more dispersed.

A detector generates a measurable signal which is usually printed out as a peak on the chromatogram. The chromatogram is a record of detector output Vs time as the analyte passes through the chromatography system. It usually consists of a series of several peaks corresponding to the different times in which components of the analyte mixture emerge from the column. The number of peaks corresponds to the minimum number of different substances (compounds or ions) contained in the analyte. If the analyte is found to display only a single peak, it is an indication that it is composed of only a single component, i.e., it is pure, although rigorous confirmation of purity may require additional testing.

### Standard conditions

The eluent used for the cation analysis is made up of 2.6 mL conc. methanesulphonic acid (MSA) in 2 L of ultra-pure water with a flow rate of 1 mL/min. The eluent used for the anion analysis is a 2.7mM  $\text{Na}_2\text{CO}_3$ /0.3mM  $\text{NaHCO}_3$  solution with a flow rate of 1.25 mL/min.

## Appendix VIII

### Dissolved inorganic nitrogen and dissolved organic nitrogen

The Skalar SAN<sup>PLUS</sup> System is a segmented continuous flow analyser. Automated segmented flow analysis is a continuous flow method of chemical analysis in which a stream of reagents and samples, segmented with air bubbles, is pumped through a manifold to undergo treatment such as mixing, heating, dialysis, etc, before entering a flow cell to be detected. Air segmentation is used to eliminate cross contamination and to provide an aliquot to mix different reagents.

#### Total Nitrogen UV digestion

The sample is mixed with a borax buffer ( $\text{Na}_2\text{B}_4\text{H}_2, 10\text{H}_2\text{O}$ ). After mixing, an excess of potassium persulfate solution is added and the mixture is digested in a UV-digester. Nitrate ( $\text{NO}_3^-$ ) is determined by the Griess Reaction after reduction of  $\text{NO}_3^-$  to nitrite by cadmium copper reduction. The colour is measured at 540 nm. For interferences see nitrate section.

#### Ammonia-N

In a buffered alkaline solution, ammonia is chlorinated to monochloramine (using dichloroisocyanurate as the chlorine source), which reacts with salicylate to 5-aminosalicylate. After oxidation and oxidative coupling, a green coloured complex is formed. The absorption of the formed complex is measured at 660 nm. This method is used for the determination of ammonia-N in waters, drainage waters, soil solutions and soil extracts ( $\text{KCl}$  and  $\text{K}_2\text{SO}_4$ ). Precipitation of calcium and magnesium hydroxides can be eliminated by the use of potassium sodium tartrate ( $\text{C}_4\text{H}_4\text{O}_6\text{KNa}, \text{H}_2\text{O}$ ) in the working buffer. Turbid samples should be filtered before determination.

### Nitrate-N

The sample is passed through a column containing granulated copper-cadmium to reduce the nitrate to nitrite. The nitrite (originally present + reduced nitrate) is determined by diazodising with sulphanilamide and coupling with  $\alpha$  - naphthylenediamine dihydrochloride (NEDD) to form a highly coloured pink azo dye which is measured at 540 nm. Iron, copper and other metals present in the sample may give negative results on  $\text{NO}_3^-$  values. Addition of 1g of  $\text{EDTA Na}_2$  per litre of buffer solution will overcome this problem, however, using imidazole ( $\text{C}_3\text{H}_4\text{N}_2$ ) as the buffer may eliminate these interferences. Turbid samples should be filtered before determination.



## Appendix IX

### Dissolved organic carbon

To measure the carbon, the carbon atoms must be converted to a measurable substance, CO<sub>2</sub>, which can then be measured by an infra-red CO<sub>2</sub>-detector. The CO<sub>2</sub> is determined by chemical and thermal oxidation of the carbon. The reaction takes place in a vessel inside an electric oven set at 670°C. The reaction vessel contains a beaded platinum catalyst to make the process more efficient.

A constant gas stream (carrier gas: CO<sub>2</sub>-free air) flows through the reaction vessel and transports the CO<sub>2</sub> to the infra-red gas analyser (CO<sub>2</sub> detector) which continuously measures the CO<sub>2</sub> concentration in the gas stream. This gas serves as the oxygen source. The amount of CO<sub>2</sub> produced is proportional to the carbon concentration of the sample. The detector measures the electric signal given by the CO<sub>2</sub> from the sample and converts it into peak area. During the oxidation, the CO<sub>2</sub> concentration plotted against time follows a typical curve, called a 'peak'. Once the sample is injected, the CO<sub>2</sub> concentration rises and quickly reaches a maximum level, then slowly drops back to the initial value. The more CO<sub>2</sub> present, the larger the peak area. The peak shape depends upon oxidation rate, which in turn depends on the substances measured and injection characteristics. Substances which oxidise quickly produce a high, slim peak, whilst substances which oxidise slowly produce lower peaks which take a longer time to fall back to the base line.

The carbon analyser then compares the peak area of the CO<sub>2</sub> from the sample with peak areas obtained from the standards used to calibrate the machine to give a reading of carbon concentration in mg/L.

### Calibration

To determine the carbon content of a sample the carbon analyser needs to be calibrated with a range of standard carbon solutions, in our case 40, 30, 20, 10 and 4 mg L<sup>-1</sup>. A linear calibration is obtained representing different peak areas, which correspond to specific carbon concentrations in mg L<sup>-1</sup>. Comparison of the peak area from the sample is then made to determine the concentration of carbon in the sample in mgC L<sup>-1</sup>.

To prepare these standard solutions, a 200 mgC L<sup>-1</sup> stock solution is used. The TOC200 carbon analyser will automatically prepare the range of standard carbon solutions that are required when instructed to do so.

### Sample preparation

The samples were soil solution from the ceramic samplers installed *in situ* stored in the freezer. Once the calibration is done, the vials were half filled with sample and put on the tray with a standard every 10 or 20 sample.

If after analysis, the samples C values are out of range, the samples were diluted with deionised water.

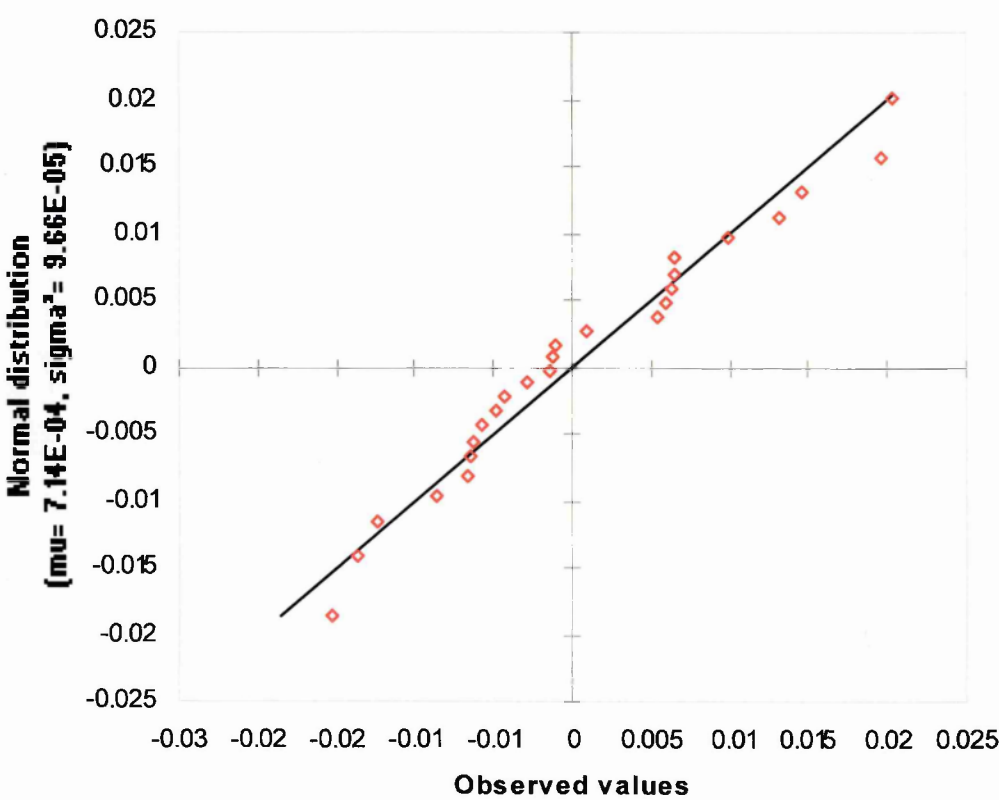
### Sparge & acidify

Because the non-purgeable organic carbon (NPOC) is desired in this study, the inorganic carbon must first be driven out of the solution. The inorganic C is in the carbonate form (Ca carb, Mg carb) which reacts with the acid and is converted to CO<sub>2</sub> which is then driven off by sparging with O<sub>2</sub>. Acidified samples won't allow any atmospheric CO<sub>2</sub> to dissolve in the samples. 20% (v/v) of HCl acid (45 µL to 2 mL) is added to change the pH of the solution, and then the sample is sparged for 90 seconds with zero-air. Once this has been done, NPOC can be measured.

# Appendix X

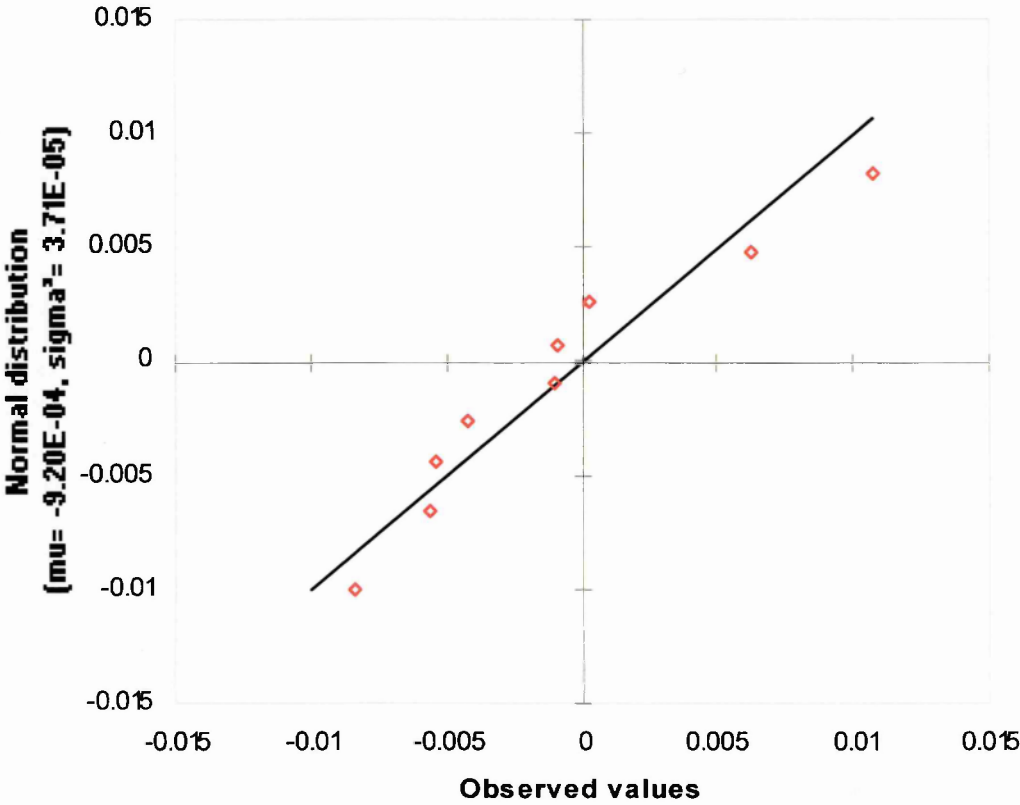
Modelling of N<sub>2</sub>O flux: individual dataset

Normal Q-Q plot



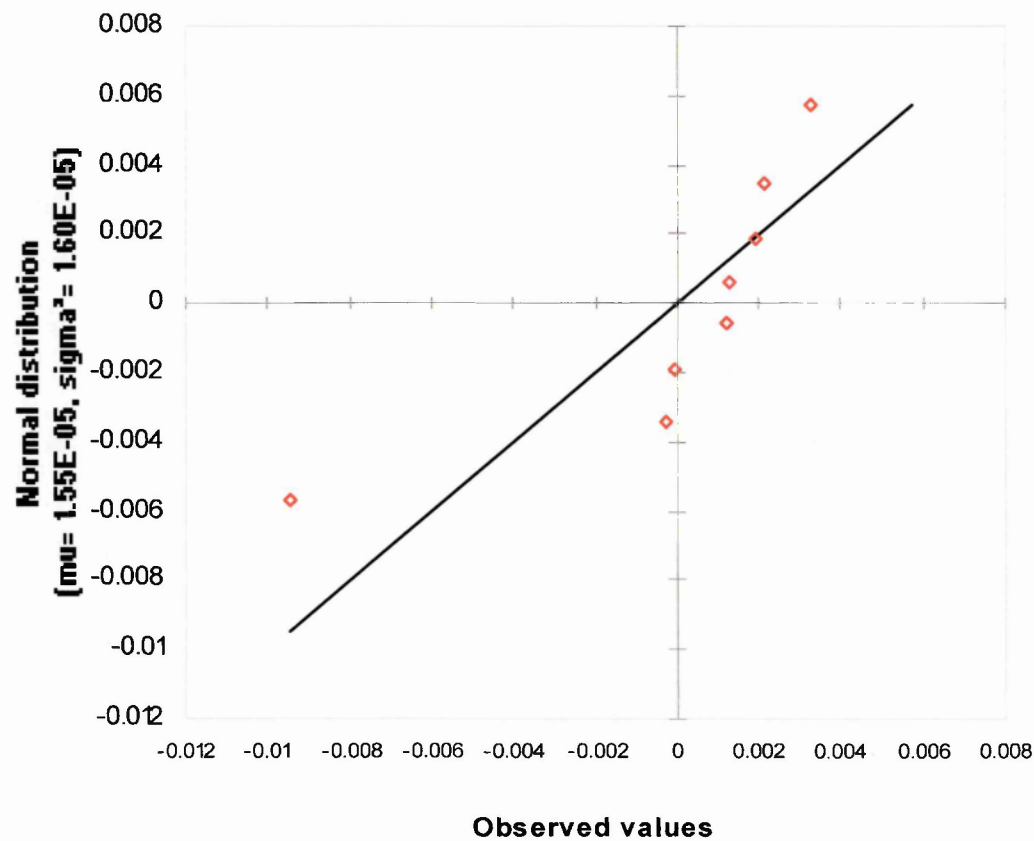
Modelling of N<sub>2</sub>O flux: mean-by-chamber dataset

Normal Q-Q plot



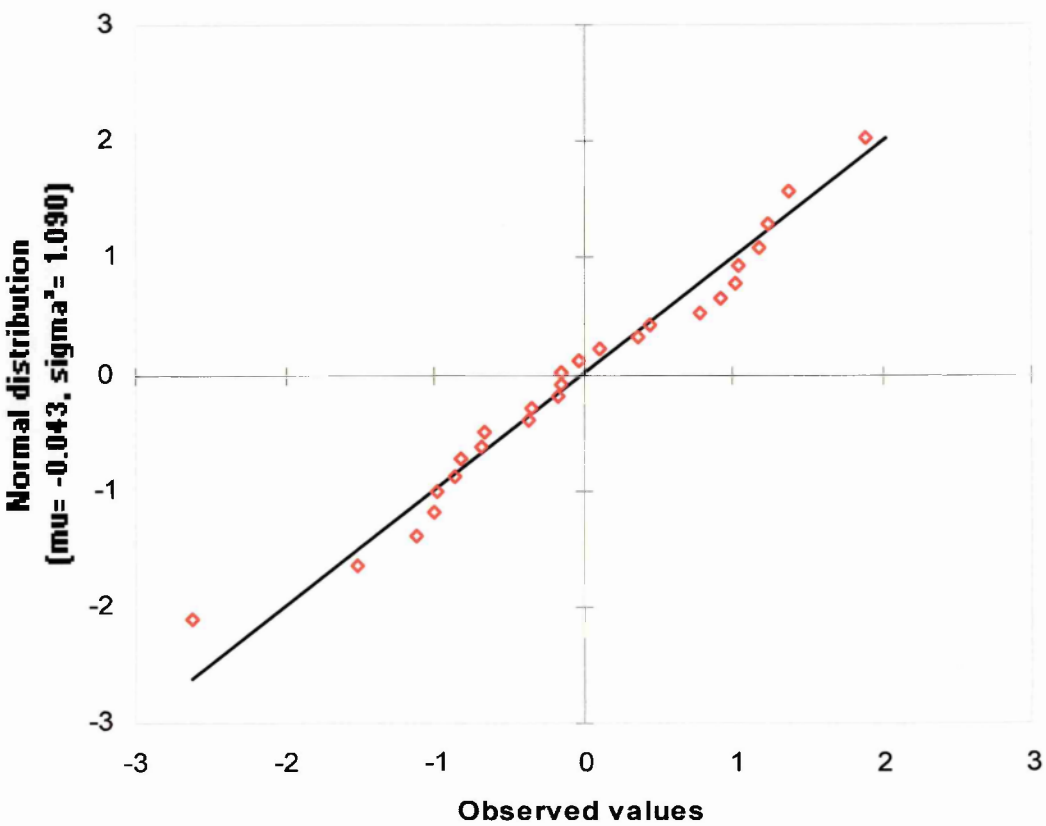
Modelling of N<sub>2</sub>O flux: mean-by-level dataset

Normal Q-Q plot

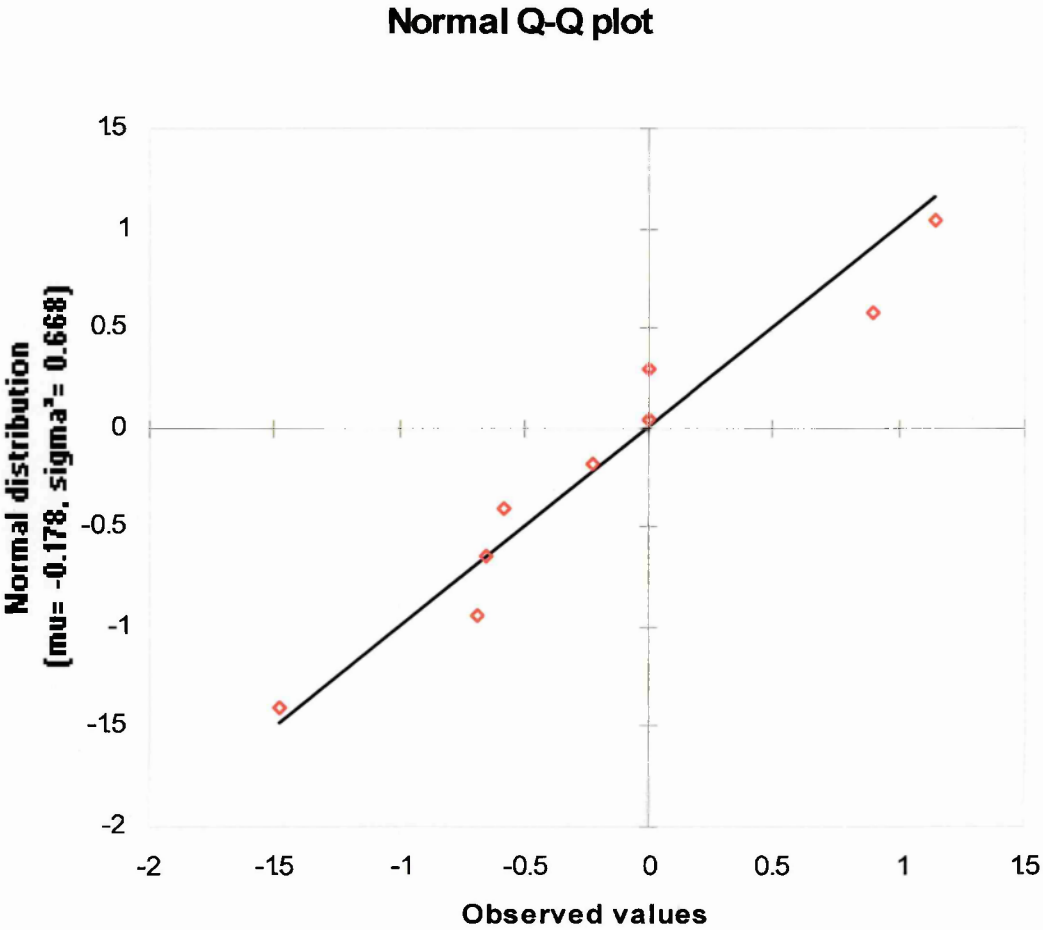


Modelling of Ln (Denitrification): individual dataset

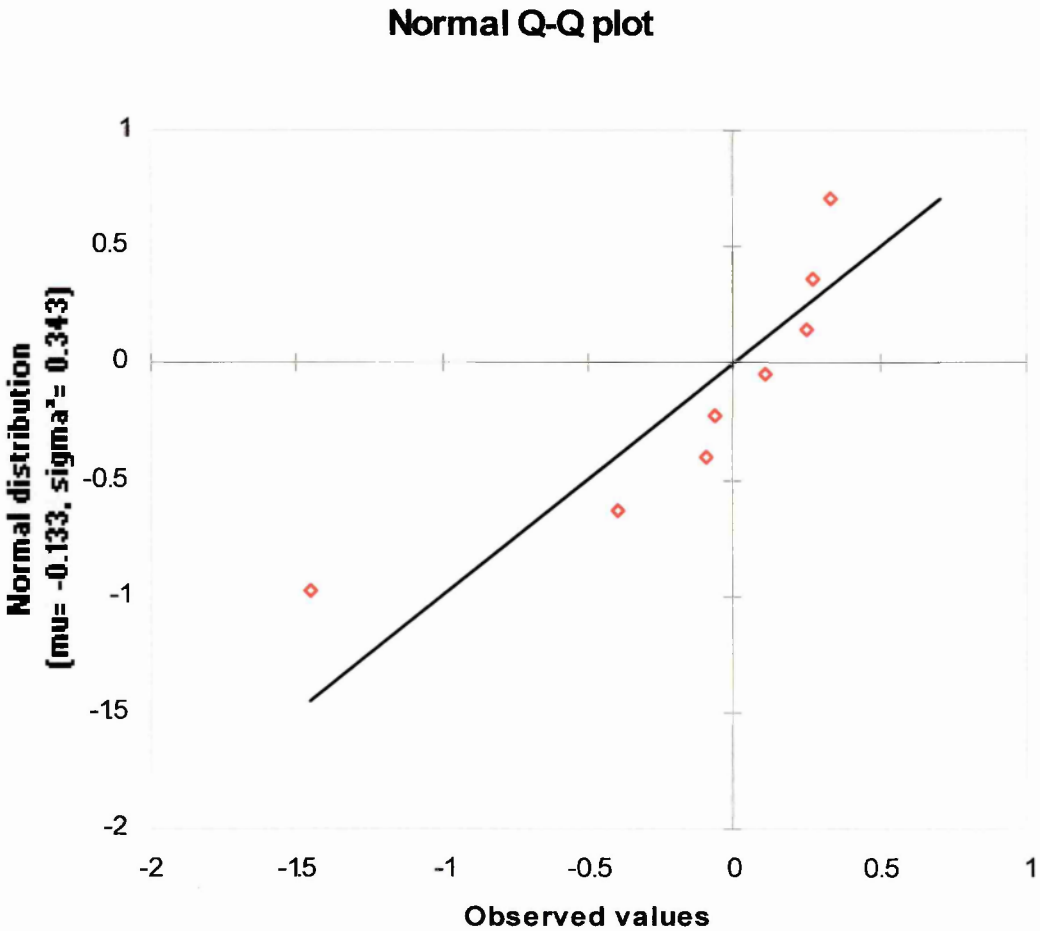
Normal Q-Q plot



Modelling of Ln (Denitrification): mean-by-chamber dataset



Modelling of Ln (Denitrification): mean-by-level dataset





## Appendix XI

(a) Estimated parameter values for each land use category

Parameter	Unit	Forest	Short Vegetation (Ungrazed)	Short Vegetation Grazed, Unfertilised (unimproved grassland)	Short Vegetation Fertilised (improved grassland)	Arable	Urban
NO <sub>3</sub> -N addition (fertiliser/livestock)	kg-N /ha/yr	0	0	0	53	97	0
NH <sub>4</sub> -N addition (fertiliser/livestock)	kg-N /ha/yr	0	0	0	53	97	0
Plant growth start day	day	60	60	60	60	60	0
Plant growth period	days	245	245	245	245	124	0
Fertiliser addition start day	day	0	0	0	60	60	0
Fertiliser addition period	days	0	0	0	184	130	0

(b) Calibrated parameters for each land use category

Parameter	Unit	Forest	Short Vegetation (Ungrazed)	Short Vegetation Grazed, Unfertilised (unimproved grassland)	Short Vegetation Fertilised (improved grassland)	Arable	Urban
Denitrification rate	day <sup>-1</sup>	0.001	0.001	0.001	0.001	0.001	0.001
Plant NO <sub>3</sub> -N uptake rate	day <sup>-1</sup>	0.05	0.05	0.05	0.03	0.03	0.05
Max. plant nitrogen uptake	kg-N/ha/yr	50	40	45	105	95	0
NH <sub>4</sub> -N nitrification rate	day <sup>-1</sup>	0.005	0.09	0.05	0.06	0.06	0
NH <sub>4</sub> -N mineralisation rate	day <sup>-1</sup>	0.1	0.1	0.1	0.2	0.2	0.1
NH <sub>4</sub> -N immobilisation rate	day <sup>-1</sup>	0.01	0.02	0.05	0.1	0.1	0
Plant NH <sub>4</sub> -N uptake rate	day <sup>-1</sup>	0.1	0.08	0.5	0.03	0.03	0
Max. Soil Moisture Deficit	mm	90	90	90	90	90	90
Max. air-soil temperature difference	°C	4.5	4.5	4.5	4.5	4.5	4.5
Soil water time constant	days	2	2	2	2	2	2
Ground water time constant	days	80	80	80	80	80	80

(c) Calibrated initial conditions									
Parameter	Unit	Forest	Short Vegetation (Ungrazed)	Short Vegetation Grazed, Unfertilised (unimproved grassland)	Short Vegetation Fertilised (improved grassland)	Arable	Urban		
Surface NO <sub>3</sub> -N concentration	mg-N/L	1	1	1	1	1	1		
Subsurface NO <sub>3</sub> -N concentration	mg-N/L	1	1	1	1	1	1		
Surface NH <sub>4</sub> -N concentration	mg-N/L	0	0	0	0	0	0		
Subsurface NH <sub>4</sub> -N concentration	mg-N/L	0	0	0	0	0	0		

# Appendix XII

(a) Estimated parameter values for each land use category

Parameter	Unit	Forest	Short Vegetation (Ungrazed)	Short Vegetation Grazed, Unfertilised (unimproved grassland)	Short Vegetation Fertilised (improved grassland)	Arable	Urban	Riparian
NO <sub>3</sub> -N addition (fertiliser/livestock)	kg-N /ha/yr	0	0	0	0	143.4	0	0
NH <sub>4</sub> -N addition (fertiliser/livestock)	kg-N /ha/yr	0	0	0	0	286.6	0	0
Plant growth start day	day	60	60	60	60	60	0	60
Plant growth period	days	245	245	245	245	124	0	124
Fertiliser addition start day	day	0	0	0	60	60	0	60
Fertiliser addition period	days	0	0	0	184	130	0	130

(b) Calibrated parameters for each land use category

Parameter	Unit	Forest	Short Vegetation (Ungrazed)	Short Vegetation Grazed, Unfertilised (unimproved grassland)	Short Vegetation Fertilised (improved grassland)	Arable	Urban	Riparian
Denitrification rate	day <sup>-1</sup>	0.001	0.001	0.001	0.001	0.001	0.001	0.5
Plant NO <sub>3</sub> -N uptake rate	day <sup>-1</sup>	0.05	0.05	0.05	0.03	0.03	0.05	0.03
Max. plant nitrogen uptake	kg-N/ha/yr	50	40	45	105	95	0	95
NH <sub>4</sub> -N nitrification rate	day <sup>-1</sup>	0.005	0.09	0.05	0.06	0.06	0	0.06
NH <sub>4</sub> -N mineralisation rate	day <sup>-1</sup>	0.1	0.1	0.1	0.2	0.2	0.1	0.2
NH <sub>4</sub> -N immobilisation rate	day <sup>-1</sup>	0.01	0.02	0.05	0.002	0.001	0	0.001
Plant NH <sub>4</sub> -N uptake rate	day <sup>-1</sup>	0.1	0.08	0.5	0.03	0.03	0	0.01
Max. Soil Moisture Deficit	mm	90	90	90	90	90	90	90
Max. air-soil temperature difference	°C	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Soil water time constant	days	4.5	2.3	2.3	2.3	2	2.3	2.3
Ground water time constant	days	150	150	150	150	150	150	150

(c) Calibrated initial conditions

Parameter	Unit	Forest	Short Vegetation (Ungrazed)	Short Vegetation Grazed, Unfertilised (unimproved grassland)	Short Vegetation Fertilised (improved grassland)	Arable	Urban	Riparian
Surface NO <sub>3</sub> -N concentration	mg-N/L	1.5	1.5	1.5	12.5	15	4	0.05
Subsurface NO <sub>3</sub> -N concentration	mg-N/L	5	5	5	5	15	5	7
Surface NH <sub>4</sub> -N concentration	mg-N/L	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Subsurface NH <sub>4</sub> -N concentration	mg-N/L	0.01	0.01	0.01	0.01	0.01	0.01	0.01

# Observed nitrate and ammonium stream concentrations used with INCA-N Riparian

\*\*\*\*\* Reach 1 \*\*\*\*\*

----- NITRATE -----

16/01/2002	11.86
31/01/2002	13.16
08/02/2002	12.13
15/02/2002	12.07
18/02/2002	11.8
19/02/2002	11.31
21/02/2002	11.13
05/03/2002	10.72
26/03/2002	8.03
02/04/2002	7.5
17/04/2002	12.8
03/05/2002	10.62
09/05/2002	9.25
16/05/2002	5.96
23/05/2002	8.75
29/05/2002	6.39
13/06/2002	6.64
18/06/2002	9.73
04/07/2002	6.09
30/07/2002	10.81
07/08/2002	3.45
15/08/2002	5.68
11/09/2002	6.53
16/10/2002	27.82
19/11/2002	18.58

\*\*\*\*\* Reach 1 \*\*\*\*\*

----- AMMONIUM -----

16/01/2002	0
31/01/2002	0.03
08/02/2002	0.09
15/02/2002	0
18/02/2002	0
19/02/2002	0
21/02/2002	0.02
05/03/2002	0.03
26/03/2002	0
02/04/2002	0.4
17/04/2002	0
03/05/2002	0
09/05/2002	0.00
16/05/2002	0
23/05/2002	0
29/05/2002	0
13/06/2002	0.05
18/06/2002	0.04
04/07/2002	0
30/07/2002	0
07/08/2002	0.08
15/08/2002	0.05
11/09/2002	0.03
16/10/2002	0
19/11/2002	1.85